Pathology of breast and ovarian cancers among *BRCA1* and *BRCA2* mutation carriers: results from the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA)

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Abstract

Background—Previous small studies found that *BRCA1* and *BRCA2* breast tumors differ in their pathology. Analysis of larger datasets of mutation carriers should allow further tumor characterization.

Methods—We used data from 4,325 *BRCA1* and 2,568 *BRCA2* mutation carriers to analyze the pathology of invasive breast, ovarian and contralateral breast cancers.

Results—There was strong evidence that the proportion of estrogen receptor (ER)-negative breast tumors decreased with age at diagnosis among *BRCA1* (*p-trend*=1.2×10^{-5}) but increased with age at diagnosis among *BRCA2* carriers (*p-trend*=6.8×10^{-6}). The proportion of triple negative tumors decreased with age at diagnosis in *BRCA1* carriers but increased with age at diagnosis of *BRCA2* carriers. In both *BRCA1* and *BRCA2* carriers, ER-negative tumors were of higher histological grade than ER-positive tumors (Grade 3 vs. Grade 1, p=1.2×10^{-13} for *BRCA1* and p=0.001 for *BRCA2*). ER and progesterone receptor (PR) expression were independently associated with mutation carrier status (ER-positive odds ratio (OR) for *BRCA2*=9.4, 95%CI: 7.0-12.6 and PR-positive OR=1.7, 95%CI:1.3-2.3, under joint analysis). Lobular tumors were more likely to be *BRCA2*-related (OR for *BRCA2*=3.3, 95%CI:2.4-4.4, p=4.4×10^{-14}), and medullary tumors *BRCA1*-related (OR for *BRCA2*=0.25, 95%CI:0.18-0.35, p=2.3×10^{-15}). ER-status of the first breast cancer was predictive of ER-status of asynchronous contralateral breast cancer (p=0.0004 for *BRCA1*; p=0.002 for *BRCA2*). There were no significant differences in ovarian cancer morphology between *BRCA1* and *BRCA2* carriers (serous:67%; mucinous:1%; endometriod:12%; clear-cell:2%).

Conclusions/Impact—Pathology characteristics of *BRCA1* and *BRCA2* tumors may be useful for improving risk prediction algorithms and informing clinical strategies for screening and prophylaxis.

Keywords

pathology; breast; *BRCA1*; *BRCA2*; contralateral

Introduction

The tumor suppressor genes *BRCA1* and *BRCA2* are associated with high risks of breast, ovarian and contralateral breast cancer. Tumors arising in *BRCA1* and *BRCA2* mutation carriers display characteristic pathological features (1-3). Cancers occurring among *BRCA1* carriers are more frequently classified as medullary (1, 4, 5) and exhibit higher grade and

mitotic count than sporadic controls (2, 6-8). Numerous studies have linked the estrogen receptor (ER)-negativity of breast tumors with *BRCA1* mutation carrier status (6, 9-16). Tumors arising in *BRCA1* carriers also tend to lack progesterone receptors (PR) and HER2, and hence display the "triple negative" (TN) phenotype (6, 10). The majority of *BRCA1* tumors express basal cytokeratins (17) and fall into the 'basal' subtype in gene expression studies (18).

Breast cancers arising in *BRCA2* mutation carriers tend to be more heterogeneous than those arising in *BRCA1* mutation carriers (19). They exhibit higher grade than tumors from age-matched sporadic controls (6, 7, 20). Several investigators have reported similar prevalence of ER-positive tumors in *BRCA2* carriers compared with sporadic controls (6, 9, 16, 17), although in one study (20) *BRCA2* tumors were more often ER-positive. *BRCA2* tumors are less likely to be HER2 overexpressing/amplified compared with sporadic tumors (6, 17). However, most studies of *BRCA2* mutation carriers have been small and detailed tumor pathology information from *BRCA2* carriers has been sparse.

Similarly, pathology studies conducted on contralateral breast cancer (21) and ovarian cancer (22-24) arising in *BRCA1* and *BRCA2* mutation carriers have been small in size. Here we report pathology data from the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA), which is the largest collaborative study of *BRCA1* and *BRCA2* mutation carriers of its kind. We assessed the morphology, grade and pathological markers in breast tumors arising in *BRCA1* or *BRCA2* carriers, and estimated age-specific distributions of the different disease subtypes. Where possible, these distributions were compared with published data for the general population. We also compared the pathology of tumors arising in *BRCA1* and *BRCA2* mutation carriers in order to identify characteristics that could distinguish between *BRCA1* and *BRCA2* mutation carriers. Such information is relevant for developing algorithms that predict mutation carrier status or breast cancer risk. For ovarian cancer, we assessed grade and morphological features of tumors. For contralateral breast cancer, we examined the relationship between pathology of the second and first invasive breast cancer. The size of the present dataset allowed the study of smaller subsets of disease, such as ER-positive tumors in *BRCA1* mutation carriers or TN tumors in *BRCA2* mutation carriers and the estimation of age-specific proportions of tumor subtypes in *BRCA1* and *BRCA2* mutation carriers, which are currently imprecise. One of the aims of this work was to replicate findings based on single reports or much smaller studies. The results of these analyses should be useful for improving breast cancer risk prediction algorithms and may inform screening practices for, and prophylaxis of, cancers arising in *BRCA1* and *BRCA2* mutation carriers.

Materials and Methods

Study participants

Eligibility to CIMBA is restricted to female *BRCA1* or *BRCA2* pathogenic mutation carriers who are 18 years or older (25). Thirty-seven groups from North America, Australia and Europe submitted data for this analysis (Supplementary Table 1). Information collected included year of birth, age at diagnosis of breast and/or ovarian cancer, age at last observation, family membership, race/ethnicity and information on bilateral prophylactic mastectomy and oophorectomy. All centers obtained informed consent from study participants and local ethical review committees approved protocols.

The present analysis was restricted to mutation carriers who had been diagnosed with breast or ovarian cancer for whom information on tumor pathological characteristics was available, and to women of self-reported white European ancestry. The number of mutation carriers of non-European ancestry with data on tumor pathology was too small to allow a meaningful

analysis. Information on at least one tumor characteristic was available for 4,325 *BRCA1* and 2,568 *BRCA2* mutation carriers.

Tumor pathology data

Pathology data were derived from medical, pathology or tumor registry records or confirmed by pathological review. For some cases, tumor pathology was based on immunohistochemical staining and scoring of tissue microarrays (TMAs). The source of the data collected by each center is shown in Supplementary Table 1. For approximately 1,000 cases detailed information on breast cancer pathology, for example staining intensity or proportion of cells staining, accompanied the summary result in the pathology records. This information was cross-checked against the marker status provided. In case of any discrepancies, the most widely used definitions for the receptor status shown in Supplementary Table 2 were used. Grades 1, 2 and 3 represent well-differentiated, moderately differentiated, and poorly/undifferentiated tumors, respectively. However, no information was available on the tumor grading system used at each center. Data were analyzed by individual hormone receptor status, joint expression of ER and PR, and HER2.

Mutation class and position

Mutations in the *BRCA1* and *BRCA2* genes can be classified according to their potential functional effect (26-28). Class 1 mutations are loss-of-function mutations, expected to result in a reduced transcript or protein level due to mRNA nonsense-mediated decay and/or degradation or instability of truncated proteins, translation reinitiation but no production of stable protein, or the absence of expression because of the deletion of transcription regulatory regions. Class 2 mutations are those likely to generate potentially stable mutant proteins that might have dominant negative action, partially preserved normal function, or loss of function. Class 2 mutations include missense substitutions, in-frame deletions and insertions, as well as truncating mutations with premature stop codons occurring in the last exon. Mutations whose consequences at the transcript or protein level could not be inferred were not considered for this classification.

Mutations occurring in the central portion of the *BRCA2* gene (NG_012772), previously referred to as the "ovarian cancer cluster region" (OCCR), are associated with a higher ratio of ovarian: breast cancer versus mutations outside this region (29, 30). We used the definition of the OCCR determined by Thompson et al. (30), as bounded by nucleotides corresponding to regions c.2831-c.3847, and c.6275-c.6401 according to the HGVS nomenclature. As there is uncertainty in defining precise boundaries, the wider region c. 2831-c.6401 was used.

Statistical methods

Logistic regression was used to assess the association between pathological characteristics and *BRCA* mutation carrier status. For assessment of continuous or ordered variables such as grade and age, tests for trend were also performed. When comparing the pathological characteristics of *BRCA1* and *BRCA2* mutation carriers, cases from countries where the mutation carriers had a mutation exclusively in either *BRCA1* or *BRCA2* (e.g., Iceland) were excluded. All analyses were adjusted for age at diagnosis and for country of origin. A robust variance approach was used to allow for dependencies between related individuals. All analyses were performed in Stata v10.

Results

Pathological characteristics of breast tumors arising in BRCA1 and BRCA2 mutation carriers

The analysis was based on 3,797 *BRCA1* mutation carriers and 2,392 *BRCA2* mutation carriers diagnosed with invasive breast cancer. Median age at diagnosis of invasive breast cancers was 40 years (interquartile range (IQR): 12.3) among *BRCA1* and 43 years (IQR: 13) among *BRCA2* mutation carriers. The majority of invasive breast cancers arising in both *BRCA1* and *BRCA2* carriers were ductal/no special-type carcinomas (Table 1). 78% of tumors arising in *BRCA1* carriers were ER-negative, 79% were PR-negative, 90% HER2-negative and 68% TN. 23% of tumors arising in *BRCA2* mutation carriers were ER-negative, 35% were PR-negative, 87% were HER2-negative, and 16% were triple negative.

Age-specific proportions of invasive breast tumors arising in *BRCA1* and *BRCA2* mutation carriers which were of histological Grade 1, Grade 2, or Grade 3 are shown in Figure 1. The number of women included in these analyses is shown in Supplementary Table 3. Age at diagnosis was associated with grade in *BRCA1* mutation carriers, in whom grade decreased with increasing age (ordered logistic regression, p-trend=1.4×10^{-15}). There was no evidence of a similar trend among *BRCA2* mutation carriers (ordered logistic regression, p-trend=0.07), however, the ratio of Grade 1 to Grade 3 tumors increased with increasing age (p=6×10^{-5}).

Age-specific proportions of ER-negative, PR-negative, HER2-negative and TN invasive breast tumors arising in *BRCA1* and *BRCA2* mutation carriers are shown in Figure 2. The frequency of ER-negative tumors decreased with the age at breast cancer diagnosis in *BRCA1* mutation carriers (p-trend=1.2×10^{-5}), and increased with the age at diagnosis in *BRCA2* mutation carriers (p-trend=6.8×10^{-6}). The distribution of PR status showed similar trends, decreasing with age at diagnosis in *BRCA1* (p-trend = 0.02) and increasing with age at diagnosis in *BRCA2* (p-trend=6.1×10^{-5}) carriers. There was no evidence of variation in the distribution of HER2 status by age at diagnosis (p-trend=0.8 and p=0.9 for *BRCA1* and *BRCA2* mutation carriers, respectively). However, the number of tumors with HER2 information was limited at the extreme age groups. The proportion of TN tumors decreased with age at breast cancer diagnosis in *BRCA1* mutation carriers (p-trend=0.01), and increased with age at diagnosis in *BRCA2* carriers (p-trend=0.001).

The analyses above were adjusted for grade; analyses without this adjustment yielded similar results. The associations were also not confounded by calendar time of diagnosis. For example, after adjusting for 5-year cohorts based on calendar year of diagnosis, the associations between ER status and age at diagnosis was still significant for both *BRCA1* and *BRCA2* mutation carriers (p-trend=2×10^{-5} and p-trend=1.2×10^{-5} respectively).

For both *BRCA1* and *BRCA2* mutation carriers there were significant differences in the distribution of tumor grade by ER status (Table 2, Figure 3) ER-negative tumors were associated with higher grade than ER-positive tumors. For example, in *BRCA1* mutation carriers, grade 3 tumors were less likely to be ER-positive than grade 1 tumors (odds ratio (OR) for ER-positivity 0.12, 95%CI:0.07-.21, p=1.2×10^{-13}). For *BRCA2* mutation carriers, grade 3 tumors were less likely to be ER-positive compared with grade 1 tumors (OR for ER-positivity, 0.33, 95%CI:0.17-0.63, p=0.001). The distribution of tumor morphology by ER status is also shown in Table 2.

We tested the hypothesis that mutation class or intragenic position influences tumor characteristics. About 66% of *BRCA1* mutation carriers harbored Class 1 mutations and 25% Class 2 mutations. Class 2 mutations were infrequent among *BRCA2* mutation carriers.

There were no significant differences between class of *BRCA1* mutation and tumor pathology of *BRCA1*-related tumors. There were also no differences in characteristics of *BRCA2*-related tumors according to whether the mutation was within the OCCR region (32% of mutation carriers) or outside the OCCR (68% of mutation carriers) (results not shown).

Information was also available on a small number of pre-invasive, ductal carcinomas in situ (DCIS). Compared with invasive breast tumors, a higher proportion of DCIS arising in *BRCA1* and *BRCA2* mutation carriers were ER-positive (Supplementary Table 4).

Comparison of BRCA1 and BRCA2 tumors

We compared the morphological characteristics of tumors arising in *BRCA1* and *BRCA2* mutation carriers. There were significantly more lobular carcinomas among *BRCA2* carriers than among *BRCA1* carriers (p=4.4×10^{-14}), and significantly more medullary or atypical medullary carcinomas among *BRCA1* mutation carriers than among *BRCA2* carriers (p=2.3×10^{-15}, Table 1).

Logistic regression analysis, treating receptor status (positive/negative) as the explanatory variable and *BRCA1/BRCA2* mutation status as the outcome variable, was used to test association between tumor characteristics and *BRCA* mutation carrier status. ER-positive tumors were more likely to arise in *BRCA2* than *BRCA1* (Table 1, OR for *BRCA2*=11.4, 95% CI: 9.8-13.2) and this was true in all morphology categories (OR for *BRCA2*=10.2, 95%CI: 8.2-12.6 among ductal/no special-type carcinoma tumors; OR=26.6, 95%CI: 4.4-159 among lobular carcinoma tumors; and OR=5.6, 95%CI: 1.8-17.2 among medullary carcinoma tumors). PR-positive (OR=6.8, 95% CI: 5.8-7.9), HER2-positive (OR=1.5, 95% CI: 1.1-2.1) and non-TN tumors (OR=11.0, 95% CI: 8.8-13.8) were also more likely to be *BRCA2* than *BRCA1* (Table 1). The associations remained significant after adjusting for tumor grade except HER2. Tumors arising in *BRCA1* mutation carriers were of significantly higher histological grade than those arising in *BRCA2* mutation carriers (Table 1). However, this difference was not significant when the analysis was adjusted for ER status.

Although ER and PR are highly correlated, expression of these hormone receptors is discordant in a small proportion of tumors. The tumors were therefore analyzed by joint ER and PR status (Supplementary Table 5). PR-positivity was associated with *BRCA2* mutation carrier status in ER-negative tumors. PR-positivity was also associated with *BRCA2* mutation carrier status in the ER-positive subset of tumors (OR for *BRCA2*=1.5, 95%CI: 1.1-2.0, p=0.01). There was no statistically significant difference in the distribution of HER2 among *BRCA1* and *BRCA2* mutation carriers when ER and/or PR positive tumors were analyzed separately, but TN tumors were significantly associated with *BRCA1* carrier status compared with HER2-positive ER-negative PR-negative tumors (Supplementary Table 5). In a model incorporating the joint effects of ER, PR and HER2 in predicting *BRCA2* vs. *BRCA1* mutation carrier status, both ER and PR remained significant, but HER2 status was not (ER: OR=9.4, 95%CI:7.0-12.6; PR: OR=1.7, 95%CI:1.3-2.3; HER2, OR=1.1, 95%CI: 0.7-1.6).

Pathological characteristics of first and contralateral breast tumors

Pathology information was available for 720 *BRCA1* and 302 *BRCA2* mutation carriers diagnosed with invasive contralateral breast cancer (CBC). The median time interval between first breast cancer and CBCs was 5.2 years (inter-quartile range (IQR): 7.5) for *BRCA1* and 5.0 years (IQR: 9.3) for *BRCA2* carriers. The median age at diagnosis of asynchronous CBC occurring more than 1 year after diagnosis of the first breast cancer, was 46 years (IQR: 13.6) for *BRCA1* and 51 years (IQR: 13.9) for *BRCA2* mutation carriers.

Morphology, grade, ER and PR status of the first invasive and asynchronous cancers are summarized in Supplementary Tables 6-9. For *BRCA1* mutation carriers, 91% of women with ER-negative first breast cancer developed ER-negative asynchronous CBC occurring more than a year after the first cancer, while 70% of women with ER-positive first cancer developed ER-negative asynchronous CBC. For *BRCA2* mutation carriers, 52% of women with ER-negative first cancer developed ER-negative asynchronous CBC and 12% of women with ER-positive first cancer developed ER-negative asynchronous CBC. Logistic regression analysis, treating receptor status (positive/negative) of the second cancer as the outcome variable and receptor status of the first cancer as the explanatory variable indicated that the ER status of the first breast cancer was predictive of ER status of the CBC for *BRCA1* mutation carriers (OR =5.8, 95%CI:2.8-11.7, p=1.2×10^{-6}, Supplementary Table 8) as well as for *BRCA2* mutation carriers (OR=11.0, 95%CI:4.3-28.6, p=7.8×10^{-7}). The conclusions were similar when the analyses were restricted to asynchronous contralateral cancers (OR=4.3, 95%CI:1.9-9.7, p=0.0004 for *BRCA1*, and OR=6.4, 95%CI:2.0-20.9, p=0.002 for *BRCA2* carriers, Supplementary Table 8). There were smaller numbers of carriers with information on both ER status and grade. When adjusted for grade the association remained significant in *BRCA1*, but was attenuated in *BRCA2* carriers, but the OR estimate was in the same direction (Supplementary Table 8). PR status of the first breast cancer was also associated with PR status of the second cancer (data not shown).

Pathology characteristics of ovarian cancers

This dataset included 838 *BRCA1* mutation carriers and 281 *BRCA2* mutation carriers who had been diagnosed with ovarian cancer. The distribution of pathological characteristics of the ovarian cancers, are shown in Table 3. The majority (67%) of all cancers (*BRCA1* and *BRCA2*) were serous. Over 70% of ovarian cancers in *BRCA1* mutation carriers were grade 3. There was no association between grade and age at cancer diagnosis (p=0.4). In *BRCA2* carriers, the proportion of grade 3 tumors increased slightly with age, whereas the proportion of grade 1 tumors decreased (ordered logistic regression, p-trend=0.03). 310 *BRCA1* and 105 *BRCA2* mutation carriers had developed breast cancer prior to ovarian cancer. History of breast cancer did not influence morphology or grade of ovarian cancer (data not shown). There were no significant differences in ovarian cancer morphology or grade between *BRCA1* and *BRCA2* mutation carriers (p>0.05, for all tests).

Discussion

We analyzed data on the pathology of breast and ovarian tumors arising in a large series of women with *BRCA1* and *BRCA2* mutations from the CIMBA consortium. Previous studies that have assessed tumor pathology in mutation carriers have been limited by small numbers, particularly among *BRCA2* carriers. The present analysis of over 4,000 *BRCA1* and 2,000 *BRCA2* carriers is the largest of its kind and allowed for accurate assessment of tumor pathology in mutation carriers, and more powerful comparisons between *BRCA1* and *BRCA2*-related tumors.

We confirmed that the majority of *BRCA1* breast cancers are ER-negative and TN tumors. We calculated age-specific proportions of tumors expressing pathological markers including ER, PR, and HER2. The proportion of ER-positive and PR-positive tumors increased with age among *BRCA1* mutation carriers, and decreased with age among *BRCA2* mutation carriers. Analyses adjusting for grade or for calendar year of diagnosis to allow for changes in screening patterns over time yielded similar results.

Tung et al (8) and Foulkes et al (12) also reported higher prevalence of ER positive tumors among *BRCA1* carriers diagnosed with breast cancer at an older age. Foulkes et al. found that at every age group the proportion of ER-negative tumours was higher in *BRCA1*

mutation carriers than non-carriers (12). We made similar observations comparing with the publicly available SEER data (31). We further confirmed differences in the distribution of grade between ER-positive and ER-negative *BRCA1* tumors previously seen in smaller studies (8, 12). Tung et al. also reported differences in pathology between sporadic ER-positive tumors and ER-positive tumors arising in *BRCA1* carriers (8). More recently, they reported that a similar percentage (80%) of ER-positive and ER-negative *BRCA1*-associated tumors showed loss of heterozygosity with loss of the wild-type *BRCA1* allele (32). They suggested that ER-positive tumors in *BRCA1* carriers could be a heterogeneous group, in some cases developing from complete loss of *BRCA1* function, while in others with intact *BRCA1* (8). Lakhani et al. further suggested that environmental exposures together with predisposition of the cells to genomic instability could result in the same cell populations producing different tumor subtypes (33). However the cell (or cells) of origin of *BRCA1*-related tumors have not been determined. These data suggest that ER-positive cancers in *BRCA1*-carriers are related to mutation carrier status rather than being incidental. A more definitive resolution of this question could be obtained by comparing the incidence of ER-positive tumors in *BRCA1* mutation carriers with the incidence of ER-positive tumors in the general population. This is not yet possible reliably, but in future prospective studies of cancer incidence should be possible to stratify analyses by tumor subtype.

Several investigators have reported similar prevalence of ER and PR positive disease in *BRCA2* carriers compared with sporadic controls (6, 9, 16, 17). Bane et al. reported higher prevalence of ER-positive tumors in a series of 64 *BRCA2* mutation carriers compared with age-matched non-carrier controls (20). We found a statistically significant decrease in the proportion of ER-positive tumors with age at diagnosis of breast cancer in *BRCA2* mutation carriers, consistent with observations in a few much smaller studies (12, 34). Compared to the publicly available SEER data from the USA, the proportion of ER-negative tumors in *BRCA2* mutation carriers appeared to be somewhat higher than that in women of the same age-group in the general population (31). Although we could not directly compare the distribution of pathological markers in mutation carriers and non-carriers in this dataset, this observation contrasts with the well-established increase in the relative incidence of ER-positive as compared with ER-negative breast cancers at older ages observed in the general population (35).

A number of risk prediction models have recently been extended to include tumor pathology information (36-38). In most of these models, variation in the expression of pathological markers with age at breast cancer diagnosis was not taken into account. Our results suggest that using age-specific pathological data in risk prediction models may provide more accurate mutation carrier predictions. Furthermore, precise characterization of the distribution of tumor pathology in mutation carriers may influence prophylactic and treatment strategies. For example, although it is known that TN status is associated with *BRCA1* mutations, in our study 16% (and up to 25% in women aged 50-60 years) of tumors in *BRCA2* mutation carriers were triple negative. Consistent with the above observations on ER status, we also found that the proportion of TN tumors in *BRCA2* carriers increased with age at diagnosis of breast cancer. This confirms the observation of Atchley et al. (10) in a smaller series, and contradicts the assumption that a diagnosis of TN disease is 'synonymous' with *BRCA1* carrier status. TN tumors would be expected to have poorer prognosis than ER-positive tumors and require chemotherapy. In addition, knowing the likelihood of developing a TN tumor in a *BRCA1* or *BRCA2* mutation carrier may influence the decision to undergo prophylactic surgery.

We confirmed that there are significant differences in the distribution of ER status between *BRCA1* and *BRCA2* breast cancers, and also found that PR is independently associated with mutation carrier status. Our results also suggest that ER-positive, PR-negative tumors were

less likely to be *BRCA2*-related than double positive tumors, and ER-negative PR-positive tumors were more likely to be *BRCA2*-related than double negative tumors. The ER-negative and PR-positive subset of tumors, previously considered a technical artefact, has now been shown in the general population to exhibit unique clinical characteristics, indicating that it is a distinct biological entity (39). We found no significant association between HER2 status and *BRCA1* or *BRCA2* mutation carrier status. However the number of HER2 overexpressing tumors may have been too small to address this reliably. TN tumors are more likely to be *BRCA1* rather than *BRCA2.* We also confirmed data showing that high grade cancers are more frequent among *BRCA1* carriers and that these women have a higher percentage of medullary cancers. By contrast, lobular cancers are substantially more frequent among *BRCA2* carriers.

We found no difference in the distribution of tumor characteristics of *BRCA1* mutation carriers by mutation category, defined by their functional effects. This analysis may be confounded by the fact that tumor characteristics may be used (together with other factors) to infer pathogenicity of a small subset of missense Class 2 *BRCA1* mutations. In this dataset only 2% of all *BRCA1* mutations would have been in this category. We also found no difference in the distribution of tumor characteristics of *BRCA2* carriers by mutation position (OCCR vs. non-OCCR). Establishing tumor pathology associated with mutations in *BRCA1* and *BRCA2* will further aid in the evaluation of unclassified variants.

We further assessed the pathology of invasive contralateral breast cancers (CBC) occurring in mutation carriers. Weitzel et al reported strong concordance in ER status between first and CBC in a study of 211 *BRCA1* and 75 *BRCA2* carriers (21). These investigators did not detect a relationship between history of Tamoxifen use or risk-reducing salphingo-oophorectomy and ER status of CBC in *BRCA1/2* mutation carriers (21). However, Swain et al found that patients in the general population with an ER-positive primary cancer receiving Tamoxifen exhibited lower concordance rate with fewer ER-positive contralateral breast cancers (40). In our study we confirmed the association between ER and PR status of first invasive breast tumors and contralateral breast cancer, suggesting that second breast cancers arising against the same genetic and environmental background are of similar pathology. However the majority (70%) of *BRCA1* mutation carriers diagnosed with ER-positive first breast cancers developed ER-negative CBC. Future CIMBA studies will aim to compare the tumor pathology of cancers occurring after RRSO.

In agreement with other reports, most ovarian cancers arising in *BRCA1* and *BRCA2* mutation carriers in our series were invasive epithelial cancers of serous histology, and we found no significant difference in morphology and grade of *BRCA1* and *BRCA2*-related tumors (22-24).

CIMBA, an international collaboration, was represented by more than 37 groups from more than 20 countries in the present study. Tumor pathology data were collected through several mechanisms, including medical records, pathology reports and TMAs. Laboratory methods for tissue preparation, immunohistochemistry and biochemical assays, scoring systems and data interpretation vary widely (Supplementary Table 1). However, data collated by CIMBA are more representative of typical assessment of pathology conducted in routine practice, and the distributions of ER and PR status across different study centers and countries in CIMBA were generally consistent. There was some variation in the distribution of HER2 status across centers. This could perhaps be explained by technical issues relating to testing of HER2 and differences in the definition of HER2 status between centers. There was also some variation in the distribution of grade and morphology across countries. Unfortunately, details of scoring for all mutation carriers were not available to standardize definitions across centers. Furthermore, data on the methods of detection of each tumor or treatment

prior to pathological analysis were not available, and these factors may influence the distribution of tumor subtypes detected (41). Future CIMBA studies will aim to collect TMA data and fixed tissue samples for rapid analysis of other markers, such as basal cytokeratins, p53, or novel candidates, and to further standardize collation of information on established markers.

A further limitation of our study is that CIMBA collects data only *BRCA1* and *BRCA2* mutation carriers. Therefore, we were unable to contrast the tumor characteristics in mutation carriers against the characteristics of breast cancers from the general population or from breast cancer patients without *BRCA1* and *BRCA2* mutations. Such an analysis would require careful selection of control subjects from the same populations who are sampled under similar conditions as *BRCA1* and *BRCA2* mutation carriers.

The genetic factors underlying aetiology of breast cancer subtypes are still not fully understood. Previous studies have reported that ER-negative tumors arising in *BRCA1* and *BRCA2* mutation carriers, presented higher genomic instability and patterns of genomic alteration than ER-positive tumors (42, 43). Many of the common breast cancer susceptibility alleles identified through GWAS are predominantly associated with either ER-positive or ER-negative disease. The pattern of association with ER-positive and ER-negative disease parallels those observed in *BRCA2* and *BRCA1* mutation carriers, respectively (44), suggesting that common mechanisms underlie the phenotype of tumors in both mutation carriers and the general population. Recently associations between the common breast cancer susceptibility alleles and separate disease subtypes in *BRCA1* and *BRCA2* mutation carriers were assessed, using data on the tumor subtype distributions presented here (Mulligan et al., In press). Mulligan et al. (In press) demonstrated differences in the associations of genetic modifiers with the risk of developing ER-positive or ER-negative breast cancer in *BRCA1* and *BRCA2* mutation carriers. These associations mirror similar differences in genetic susceptibility to ER-positive or ER-negative disease seen in the general population (45, 46) . The apparent differences in SNP associations between *BRCA1* and *BRCA2* carriers, and non-carriers observed previously, may be explained by differences in the prevalence of tumor subtypes.

The present CIMBA study is the largest of its kind, allowing more accurate characterization of the pathology of *BRCA1* and *BRCA2* tumors. As participants were collated from diverse countries and study centers, the findings should be widely applicable. We were able to calculate precise age-specific distributions of markers expressed, and replicate findings reported in only a few small studies to date. This information should be helpful for improving the performance of breast cancer risk prediction models that calculate *BRCA1* and *BRCA2* mutation carrier probabilities, or for developing algorithms that predict the risk of specific breast cancer subtypes for mutation carriers. These may be of clinical utility in guiding screening and prophylactic practices for mutation carriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Breast Cancer Family Registry (BCFR)

Breast Cancer Family Registry (BCFR) - Ontario site

Baltic Familial Breast Ovarian Cancer Consortium (BFBOCC)

Copenhagen Breast Cancer Study (CBCS)

Spanish National Cancer Center (CNIO)

Deutsches Krebsforschungszentrum (DKFZ) study.

The Hereditary Breast and Ovarian Cancer Research Group Netherlands (HEBON)

Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)

Fox Chase Cancer Center (FCCC) Fox Chase Cancer Center Biosample Repository

University of Kansas Medical Center (KUMC)

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(A)

(B)
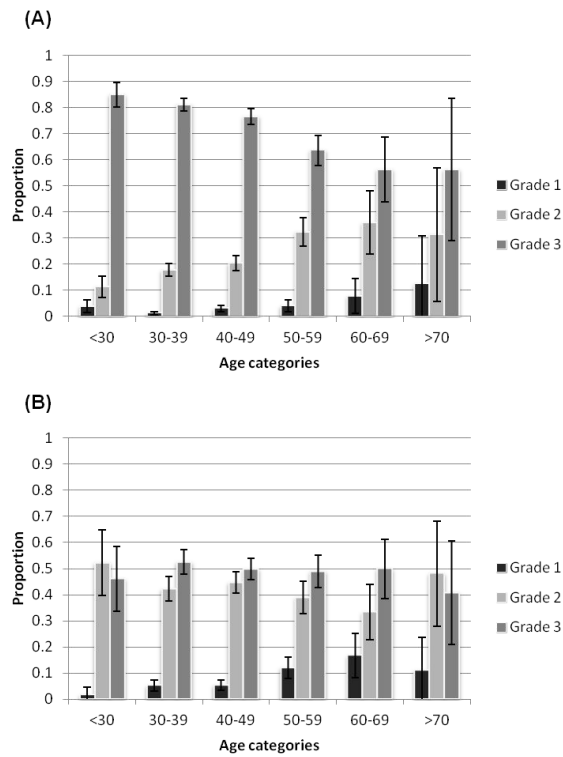
Figure 1. Age specific proportions of Grade 1, 2 and 3 breast tumors arising in *BRCA1* and *BRCA2* mutation carriers

Age specific proportions of Grade 1, Grade 2, and Grade 3 tumors among (A) *BRCA1* and (B) *BRCA2* mutation carriers.

Error bars represent robust confidence intervals associated with each proportion.
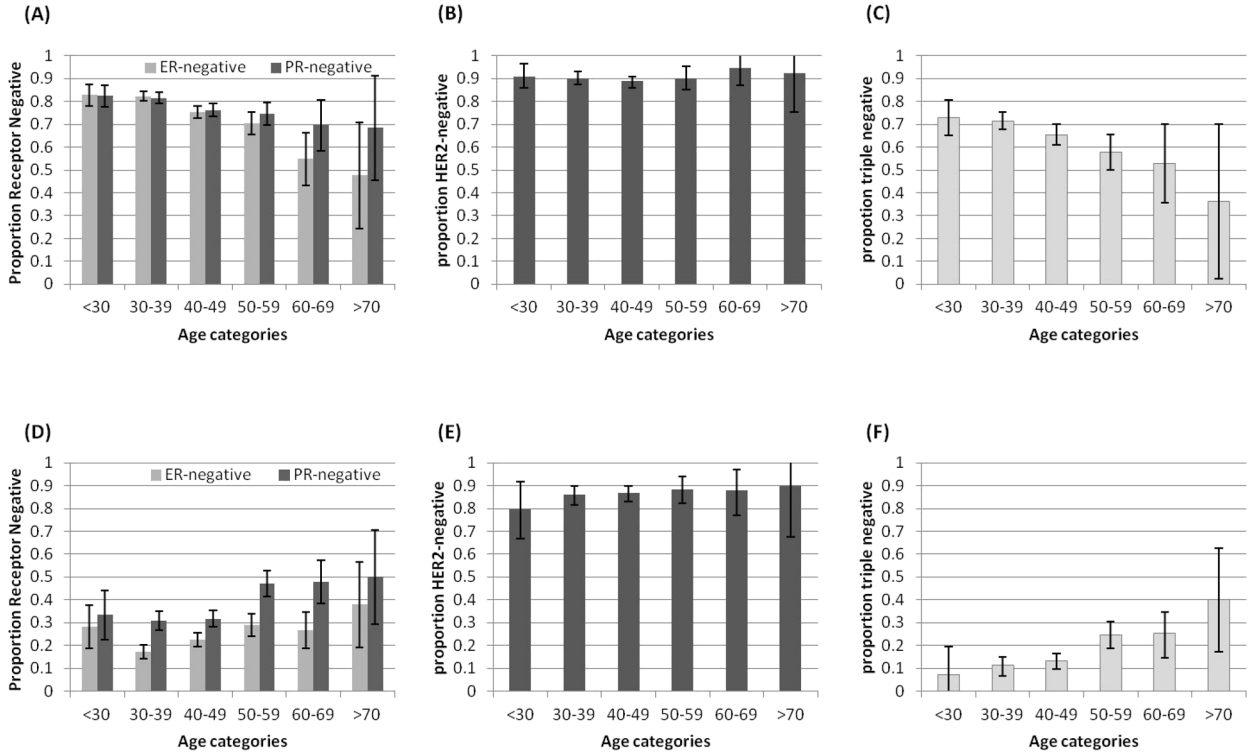
Figure 2. Age specific proportions of pathological subtypes of breast tumors arising in *BRCA1* and *BRCA2* mutation carriers

Age specific proportions of (A) Receptor-negative (ER, PR) (B) HER2-negative, and (C) triple negative tumors arising in *BRCA1* mutation carriers, and of (D) Receptor-negative (ER, PR) (E) HER2-negative, and (F) triple negative tumors arising in *BRCA2* mutation carriers. ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2. Error bars represent robust confidence intervals associated with each proportion.

(A)

(B)

(C)

(D)
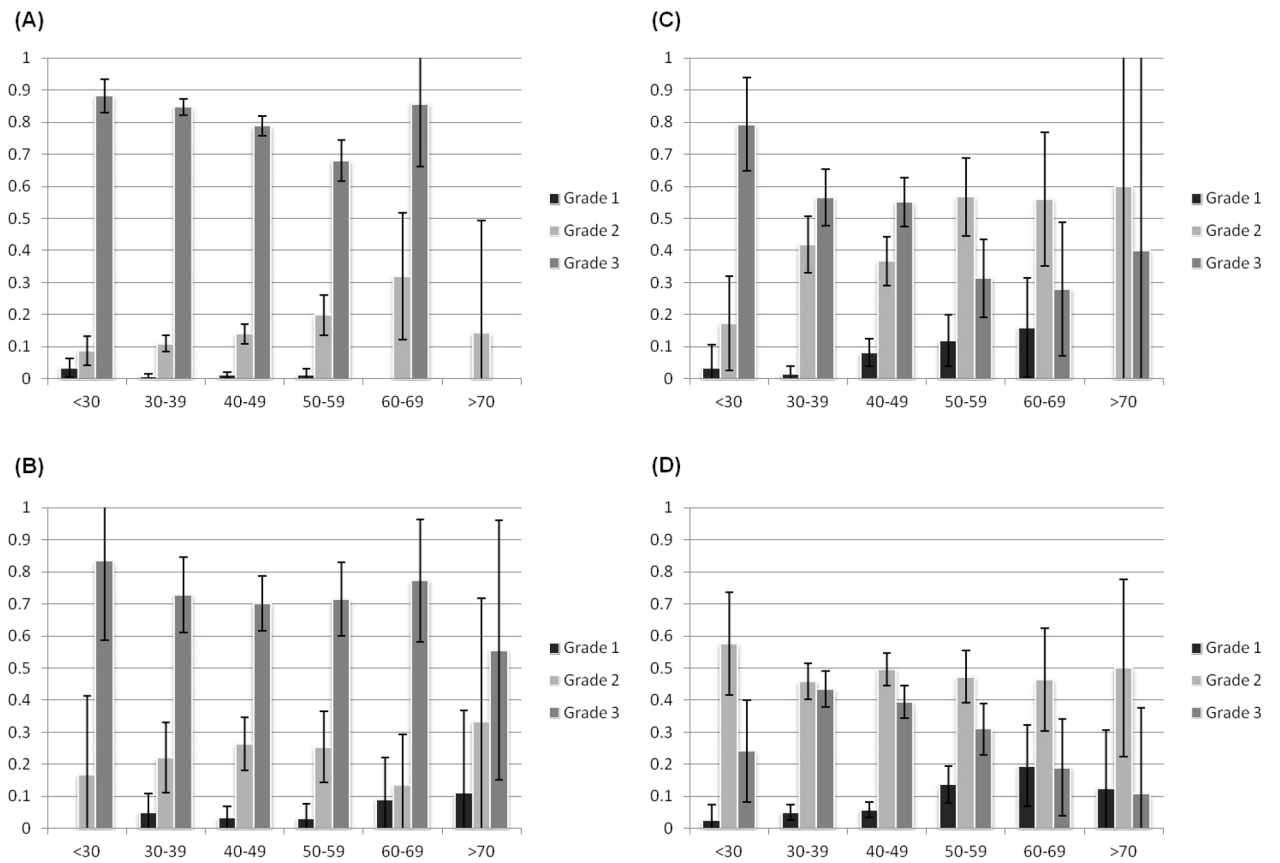
Figure 3. Distribution of Grade within ER-positive and ER-negative *BRCA1* and *BRCA2* mutation carriers

Age specific proportions of Grade 1, Grade 2, and Grade 3 (A) ER-negative, and (C) ER-positive breast tumors arising in *BRCA1* mutation carriers, and (B) ER-negative, and (D) ER-positive breast tumors arising in *BRCA2* mutation carriers.

ER, estrogen receptor

Table 1

Pathology of invasive breast cancer in *BRCA1* and *BRCA2* mutation carriers and Odds Ratios for predicting *BRCA2* mutation carrier status

|	*BRCA1* n (%)*	*BRCA2* n (%)*	OR[†]	95%CI	OR[‡]	95%CI
Morphology						
Invasive Ductal	2,387 (80)	1,515 (83)	1.00	-	-	-
Invasive Lobular	67 (2.2)	153 (8.4)	3.3	(2.4-4.4)	-	-
Medullary §	281 (9.4)	40 (2.2)	0.25	(0.18-0.35)	-	-
Other	258 (8.6)	116 (6.4)	0.81	(0.63-1.02)	-	-
ER, PR, HER2, TN						
ER-positive	625 (22)	1,475 (77)	11.4	9.8-13.2	10.0	8.2-12.1
PR-positive	539 (21)	1,084 (64)	6.8	5.8-7.9	5.5	4.5-6.6
HER2-positive	138 (10)	121 (13)	1.5	1.1-2.1	1.3	0.9-1.9
Non-TN (vs. TN)	411 (31)	700 (84)	11.0	8.8-13.8	9.0	6.8-11.8
Grade						
Grade 1	64 (3)	100 (7)	1.0	-	1.0	-
Grade 2	481 (20)	603 (43)	1.01	0.72-1.44	1.1	0.67-1.8
Grade 3	1,822 (77)	711 (50)	0.32	0.23-0.45	0.71	0.44-1.17

*
Number of tumors (n) of each morphology type or tumor grade, or number of receptor positive tumors, and as percentage (%) of all *BRCA1* or *BRCA2*-related tumors

†
Odds ratios for *BRCA2* mutation carrier status, compared with *BRCA1* mutation carrier status, associated with tumor morphology, receptor positive tumors, or Grade 2 vs. Grade 1, and Grade 3 vs. Grade 1 tumors; analyses were adjusted for country and age at diagnosis

‡
analyses adjusted for country, age at diagnosis, and tumor grade; OR for analysis of grade, adjusted for country, age at diagnosis and ER status

§
includes atypical medullary carcinomas

OR, odds ratio; CI, confidence interval; estrogen receptor, ER; progesterone receptor, PR; Human epidermal growth factor receptor 2, HER2; TN, triple negative

Table 2

Distribution of Grade and morphology of ER-positive and ER-negative tumours in *BRCA1* and *BRCA2* mutation carriers

Type	BRCA1 mutation carriers			BRCA2 mutation carriers		
	ER-negative n (%)*	ER-positive n (%)	OR† (95%CI)	ER-negative n (%)	ER-positive n (%)	OR (95%CI)
Grade						
Grade 1	18 (1.2)	28 (6.9)	1.00	12 (4.3)	68 (7.4)	1.00
Grade 2	191 (13.1)	170 (41.6)	0.57 (0.31-1.05)	67 (24)	442 (48.2)	1.2 (0.60-2.4)
Grade 3	1246 (85.7)	210 (51.5)	0.12 (0.07-0.21)	200 (71.7)	408 (44.4)	0.33(0.17-0.6)
Morphology						
Ductal	1,353 (58.8)	385(86.5)	-	266 (85.8)	889 (84)	-
Lobular	16(2.7)	21 (4.7)	-	15 (4.8)	110 (10.4)	-
Medullary §	141(23.5)	17 (3.8)	-	14 (4.6)	13 (1.2)	-
Other	91(15)	22 (5)	-	15 (4.8)	46 (4.3)	-

*
Number of tumors (n) of each grade or morphology type by ER-status as a percentage (%) of all ER-negative or ER-positive tumors where this information is available.

†
OR and 95%CI are for ER-positive vs. ER-negative disease in Grade 2 or Grade 3 tumors are compared with Grade 1 tumors. These analyses were adjusted for age at diagnosis of breast cancer and country of origin.

§
includes medullary and atypical medullary tumours.

ER, estrogen receptor; OR, odds ratio; CI, confidence intervals

Table 3

Distribution of morphology and grade of ovarian tumors arising in *BRCA1* and *BRCA2* mutation carriers

Factor		*BRCA1* n (%)	*BRCA2* n (%)	Total n (%)
Morphology	Serous	534 (66)	191 (70)	725 (67)
	Mucinous	11 (1)	4 (1)	15 (1)
	Endometrioid	94 (12)	33 (12)	127 (12)
	Clear cell	8 (1)	8 (3)	16 (1)
	Other	166 (20)	36 (13)	202 (19)
	Total	**813**	**272**	**1,085**
Grade	1	17 (3)	11 (6)	28 (4)
	2	104 (20)	37 (21)	141 (20)
	3	407 (77)	128 (73)	535 (76)
	Total	**528**	**176**	**704**

Number of tumors (n) of each morphology type or grade, and as percentage (%) of all *BRCA1* or *BRCA2*-related tumors where this information is available