Author Manuscript

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2012 July 01.

Published in final edited form as:

*Cancer Epidemiol Biomarkers Prev.* 2012 January ; 21(1): 134–147. doi: 10.1158/1055-9965.EPI-11-0775.

# 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*= $1.2 \times 10^{-5}$ ) but increased with age at diagnosis among *BRCA2* carriers (*p-trend*= $6.8 \times 10^{-6}$ ). 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= $1.2 \times 10^{-13}$  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*=0.25, 95%CI:0.18-0.35, p= $2.3 \times 10^{-15}$ ). ERstatus 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

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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 agematched 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 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 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= $1.4 \times 10^{-15}$ ). 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= $6 \times 10^{-5}$ ).

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= $1.2 \times 10^{-5}$ ), and increased with the age at diagnosis in *BRCA2* mutation carriers (p-trend= $6.8 \times 10^{-6}$ ). 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= $6.1 \times 10^{-5}$ ) 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= $2 \times 10^{-5}$  and p-trend= $1.2 \times 10^{-5}$  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= $1.2 \times 10^{-13}$ ). 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= $4.4 \times 10^{-14}$ ), and significantly more medullary or atypical medullary carcinomas among *BRCA1* mutation carriers than among *BRCA2* carriers (p= $2.3 \times 10^{-15}$ , 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 *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= $1.2 \times 10^{-6}$ , Supplementary Table 8) as well as for *BRCA2* mutation carriers (OR=11.0, 95%CI:4.3-28.6, p= $7.8 \times 10^{-7}$ ). 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 ERpositive 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 BRCA1related 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 ERpositive 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

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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 salphingooophorectomy 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 ERpositive 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 ERpositive or ER-negative disease. The pattern of association with ER-positive and ERnegative 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 ERnegative 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

This work was supported by Cancer Research UK grants C12292/A11174 and C1287/A10118. The research leading to these results has received funding from the European Community's Seventh Framework Programme under grant agreement n° 223175 (HEALTH-F2-2009-223175). NM was funded by a scholarship from the Medical Research Council. ACA is a CR-UK Senior Cancer Research Fellow. DFE is a CR-UK Principal Research Fellow.

Study specific acknowledgments:

#### Breast Cancer Family Registry (BCFR)

This work was supported by the National Cancer Institute, National Institutes of Health under RFA-CA-06-503 and through cooperative agreements with members of the Breast Cancer Family Registry and Principal Investigators, including Cancer Care Ontario (U01 CA69467), Columbia University (U01 CA69398), Fox Chase Cancer Center (U01 CA69631), Huntsman Cancer Institute (U01 CA69446), Cancer Prevention Institute of California (U01 CA69417), University of Melbourne (U01 CA69638), and Research Triangle Institute Informatics Support Center (RFP No. N02PC45022-46). Samples from the FCCC, HCI, and CPIC were processed and distributed by the Coriell Cell Repositories through cooperative agreements. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the BCFR.

#### Breast Cancer Family Registry (BCFR) - Ontario site

This work was supported by Cancer Care Ontario and the US National Cancer Institute, National Institutes of Health under RFA # CA-06-503 and through cooperative agreements with members of the Breast Cancer Family Registry (BCFR) and Principal Investigators. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the BCFR, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the BCFR. We wish to thank Gord Glendon, Teresa Selander, Mona Gill, Lucine Collins, Nayana Weerasooriya and members of the Ontario Familial Breast Cancer Registry for their contributions to the study.

#### Baltic Familial Breast Ovarian Cancer Consortium (BFBOCC)

Lithuania: This work is supported by the Research Council of Lithuania grant LIG-19/2010 to Ramunas Janavicius.

Latvia: LT was supported by LSC grants 05.0023. 04. and 10.0010.08. The Genome Database of Latvian Population, Latvian Biomedical Research and Study Center is acknowledged for providing data and DNA samples.

#### Copenhagen Breast Cancer Study (CBCS)

We thank Bent Ejlertsen for Clinical data and the NEYE Foundation for financial support.

#### Spanish National Cancer Center (CNIO)

We thank R.M. Alonso, G Pita and R.M. Milne for their assistance. This study was partially supported by Fundación Mutua Madrileña, Asociación Española Contra el Cáncer and the Spanish Ministry of Science and Innovation (FIS PI08 1120). Funded in part by the Basque Foundation for Health Innovation and Research (BIOEF): BIO07/CA/006.

#### Deutsches Krebsforschungszentrum (DKFZ) study.

The Deutsches Krebsforschungszentrum (DKFZ) study was supported by the DKFZ.

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

HEBON Collaborating Centers: Coordinating center: Netherlands Cancer Institute, Amsterdam, NL: F.B.L. Hogervorst, S. Verhoef, M. Verheus, L.J. van 't Veer, F.E. van Leeuwen, M.A. Rookus; Erasmus Medical Center, Rotterdam, NL: M. Collée, A.M.W. van den Ouweland, A. Jager, M.J. Hooning, M.M.A. Tilanus-Linthorst, C. Seynaeve; Leiden University Medical Center, NL, Leiden: C.J. van Asperen, J.T. Wijnen, M.P. Vreeswijk, R.A. Tollenaar, P. Devilee; Radboud University Nijmegen Medical Center, Nijmegen, NL: M.J. Ligtenberg, N. Hoogerbrugge; University Medical Center Utrecht, Utrecht, NL: M.G. Ausems, R.B. van der Luijt; Amsterdam Medical Center, NL: C.M. Aalfs, T.A. van Os; VU University Medical Center, Amsterdam, NL: J.J.P. Gille, Q. Waisfisz, H.E.J. Meijers-Heijboer; University Hospital Maastricht, Maastricht, NL: E.B. Gomez-Garcia, C.E. van Roozendaal, Marinus J. Blok, B. Caanen; University Medical Center Groningen University, NL: J.C. Oosterwijk, A.H. van der Hout, M.J. Mourits; The Netherlands Foundation for the detection of hereditary tumours, Leiden, NL: H.F. Vasen. The HEBON study is supported by the Dutch Cancer Society grants NK11998-1854, NK12004-3088, NK12007-3756 and the ZonMW grant 91109024.

#### Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)

Douglas Easton is the Principal Investigator of the study. EMBRACE Collaborating Centers are: Coordinating Centre, Cambridge: Susan Peock, Debra Frost, Steve Ellis, Elena Fineberg. North of Scotland Regional Genetics Service, Aberdeen: Zosia Miedzybrodzka, Helen Gregory. Northern Ireland Regional Genetics Service, Belfast: Patrick Morrison, Lisa Jeffers. West Midlands Regional Clinical Genetics Service, Birmingham: Trevor Cole, Kairen Ong, Jonathan Hoffman. South West Regional Genetics Service, Bristol: Alan Donaldson, Margaret James.

East Anglian Regional Genetics Service, Cambridge: Joan Paterson, Sarah Downing, Amy Taylor. Medical Genetics Services for Wales, Cardiff: Alexandra Murray, Mark T. Rogers, Emma McCann. St James's Hospital, Dublin & National Centre for Medical Genetics, Dublin: M. John Kennedy, David Barton. South East of Scotland Regional Genetics Service, Edinburgh: Mary Porteous, Sarah Drummond. Peninsula Clinical Genetics Service, Exeter: Carole Brewer, Emma Kivuva, Anne Searle, Selina Goodman, Kathryn Hill. West of Scotland Regional Genetics Service, Glasgow: Rosemarie Davidson, Victoria Murday, Nicola Bradshaw, Lesley Snadden, Mark Longmuir, Catherine Watt, Sarah Gibson, Eshika Haque, Ed Tobias, Alexis Duncan. South East Thames Regional Genetics Service, Guy's Hospital London: Louise Izatt, Chris Jacobs, Caroline Langman, Anna Whaite. North West Thames Regional Genetics Service, Harrow: Huw Dorkins. Leicestershire Clinical Genetics Service, Leicester: Julian Barwell. Yorkshire Regional Genetics Service, Leeds: Julian Adlard, Carol Chu, Julie Miller. Cheshire & Merseyside Clinical Genetics Service, Liverpool: Ian Ellis, Catherine Houghton. Manchester Regional Genetics Service, Manchester: D. Gareth Evans, Fiona Lalloo, Jane Taylor. North East Thames Regional Genetics Service, NE Thames, London: Lucy Side, Alison Male, Cheryl Berlin. Nottingham Centre for Medical Genetics, Nottingham: Jacqueline Eason, Rebecca Collier. Northern Clinical Genetics Service, Newcastle: Fiona Douglas, Oonagh Claber, Irene Jobson. Oxford Regional Genetics Service, Oxford: Lisa Walker, Diane McLeod, Dorothy Halliday, Sarah Durell, Barbara Stayner. The Institute of Cancer Research and Royal Marsden NHS Foundation Trust: Ros Eeles, Susan Shanley, Nazneen Rahman, Richard Houlston, Elizabeth Bancroft, Lucia D'Mello, Elizabeth Page, Audrey Ardern-Jones, Kelly Kohut, Jennifer Wiggins, Elena Castro, Anita Mitra, Lisa Robertson. North Trent Clinical Genetics Service, Sheffield: Jackie Cook, Oliver Quarrell, Cathryn Bardsley. South West Thames Regional Genetics Service, London: Shirley Hodgson, Sheila Goff, Glen Brice, Lizzie Winchester, Charlotte Eddy, Vishakha Tripathi, Virginia Attard. Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton: Diana Eccles, Anneke Lucassen, Gillian Crawford, Donna McBride, Sarah Smalley. EMBRACE is supported by Cancer Research UK Grants C1287/A10118 and C1287/A11990. D. Gareth Evans and Fiona Lalloo are supported by an NIHR grant to the Biomedical Research Centre, Manchester. The Investigators at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust are supported by an NIHR grant to the Biomedical Research Centre at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust. Ros Eeles, Elizabeth Bancroft and Lucia D'Mello are also supported by Cancer Research UK Grant C5047/A8385.

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

We acknowledge M. Pat Gilroy, Lesley Cruz, Diane Faison, Barbara Dettore, Mary Donovan, and Meghan Butler for their help collecting patient data and samples.

#### University of Kansas Medical Center (KUMC)

A.K.G. was funded by U01CA69631, 5U01CA113916, and the Eileen Stein Jacoby Fund. The author acknowledges support from The University of Kansas Cancer Center and the Kansas Bioscience Authority Eminent Scholar Program. A.K.G. is the Chancellors Distinguished Chair in Biomedical Sciences endowed Professor.

#### The German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC)

GC-HBOC is supported by a grant of the German Cancer Aid (grant 109076), the Centre of Molecular Medicine Cologne (CMMC).

#### Genetic Modifiers of cancer risk in BRCA1/2 mutation carriers (GEMO) study : Cancer Genetics Network "Groupe Génétique et Cancer", Fédération Nationale des Centres de Lutte Contre le Cancer, France

The study was supported by the Ligue National Contre le Cancer and the Association "Le cancer du sein, parlonsen" Award. We wish to thank all the GEMO collaborating groups for their contribution to this study. GEMO Collaborating Centers are: Coordinating Centres, Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Centre Hospitalier Universitaire de Lyon / Centre Léon Bérard, & Equipe «Génétique du cancer du sein», Centre de Recherche en Cancérologie de Lyon: Olga Sinilnikova, Sylvie Mazoyer, Laure Barjhoux, Carole Verny-Pierre, Sophie Giraud, Mélanie Léone; and Service de Génétique Oncologique, Institut Curie, Paris: Dominique Stoppa-Lyonnet, Marion Gauthier-Villars, Bruno Buecher, Claude Houdayer, Virginie Moncoutier, Muriel Belotti, Carole Tirapo, Antoine de Pauw. Institut Gustave Roussy, Villejuif: Brigitte Bressac-de-Paillerets, Audrey Remenieras, Véronique Byrde, Olivier Caron, Gilbert Lenoir. Centre Jean Perrin, Clermont-Ferrand: Yves-Jean Bignon, Nancy Uhrhammer. Centre Léon Bérard, Lyon: Christine Lasset, Valérie Bonadona. Centre François Baclesse, Caen: Agnès Hardouin, Pascaline Berthet. Institut Paoli Calmettes, Marseille: Hagay Sobol, Violaine Bourdon, Tetsuro Noguchi, François Eisinger. Groupe Hospitalier Pitié-Salpétrière, Paris: Florence Coulet, Chrystelle Colas, Florent Soubrier. CHU de Arnaud-de-Villeneuve, Montpellier: Isabelle Coupier, Pascal Pujol. Centre Oscar Lambret, Lille: Jean-Philippe Peyrat, Joëlle Fournier, Françoise Révillion, Philippe Vennin, Claude Adenis. Hôpital René Huguenin/Institut Curie, St Cloud: Etienne Rouleau, Rosette Lidereau, Liliane Demange, Catherine Nogues. Centre Paul Strauss, strasbourg: Danièle Muller, Jean-Pierre Fricker. Institut Bergonié, Bordeaux: Michel Longy, Nicolas Sevenet. Institut Claudius Regaud, toulouse: Christine Toulas, Rosine Guimbaud, Laurence Gladieff, Viviane Feillel. CHU de Grenoble: Dominique Leroux, Hélène Dreyfus, Christine Rebischung, Magalie Peysselon. CHU de Dijon: Fanny Coron, Laurence Faivre. CHU de St-Etienne: Fabienne

Prieur, Marine Lebrun, Caroline Kientz. Hôtel Dieu Centre Hospitalier, Chambéry: Sandra Fert Ferrer. Centre Antoine Lacassagne, Nice: Marc Frénay. CHU de Limoges: Laurence Vénat-Bouvet. CHU de Nantes: Capucine Delnatte. CHU Bretonneau, Tours: Isabelle Mortemousque. Creighton University, Omaha, USA: Henry T.Lynch, Carrie L.Snyder.

#### Georgetown University (GEORGETOWN)

CI and BNP are supported by National Cancer Institute Grant (NCI P30 CA51008-12) and by the Fisher Center for Familial Cancer Research.

#### Helsinki Breast Cancer Study (HEBCS)

HEBCS thanks Drs. Kirsimari Aaltonen and Carl Blomqvist and research nurse Irja Erkkilä for their help with the patient data. The HEBCS study has been financially supported by the Helsinki University Central Hospital Research Fund, Academy of Finland (132473), the Finnish Cancer Society, and the Sigrid Juselius Foundation.

#### Iceland Landspitali - University Hospital (ILUH)

The ILUH group was supported by the Icelandic Association "Walking for Breast Cancer Research" and by the Landspitali University Hospital Research Fund.

#### Interdisciplinary Health Research International Team Breast Cancer Susceptibility (INHERIT BRCAs)

We would like to thank Martine Tranchant for skillful technical assistance. J.S. is Chairholder of the Canada Research Chair in Oncogenetics. This work was supported by the Canadian Institutes of Health Research for the "CIHR Team in Familial Risks of Breast Cancer" program and by the Canadian Breast Cancer Research Alliance-grant #019511.

#### Istituto Oncologico Veneto - Hereditary Breast Ovarian Cancer Study (IOVHBOCS)

This study was supported by "Ministero della Salute" (grant numbers RFPS 2006-5-341353, ACC2/R6.9 and "Progetto Tumori Femminili")

#### Kathleen Cuningham Consortium for Research into Familial Breast Cancer (KCONFAB)

We wish to thank Heather Thorne, Eveline Niedermayr, all the kConFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the Clinical Follow Up Study (funded by NHMRC grants 145684, 288704 and 454508) for their contributions to this resource, and the many families who contribute to kConFab. kConFab is supported by grants from the National Breast Cancer Foundation, the National Health and Medical Research Council (NHMRC) and by the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the Cancer Foundation of Western Australia, Amanda Spurdle is supported by an NHMRC Senior Research Fellowship, and Georgia Chenevix-Trench by an NHMRC Senior Principal Research Fellowship.

#### Mayo Clinic (MAYO)

The MAYO study was supported by NIH grants CA116167, CA128978, a Specialized Program of Research Excellence (SPORE) in Breast Cancer (CA116201), and awards from the Komen Foundation for the Cure and the Breast Cancer Research Foundation.

#### Memorial Sloane Kettering Cancer Center (MSKCC)

MSKCC Acknowledgements: The Breast Cancer Research Foundation, The Niehaus, Weissenbach, Southworth Cancer Research Initiative, The Sharon Levine Corzine Research Fund, The Esther and Hyman Rapport Philanthropic Trust

#### National Cancer Institute (NCI)

The research of Drs. PL Mai and MH Greene was supported by the Intramural Research Program of the US National Cancer Institute, and by support services contracts NO2-CP-11019-50 and N02-CP-65504 with Westat, Inc, Rockville, MD.

#### N.N. Petrov Institute of Oncology (NNPIO)

This work has been supported by the Russian Federation for Basic Research (grants 10-04-92601, 11-04-00250, 11-04-00227), the Federal Agency for Science and Innovations (contract 02.740.11.0780), the Commission of the European Communiti (grant PITN-GA-2009-238132)

This work was supported by the OSU Comprehensive Cancer Center. Caroline Craven and Michelle O'Connor were responsible for patient accrual and data management.

#### Universita de Pisa (PBCS)

Maria A Caligo was supported by a ITT (Tuscany Institute for Tumors) grant 2010-2012.

#### Swedish Breast Cancer Study (SWE-BRCA)

SWE-BRCA collaborators (in Lund and Stockholm): Åke Borg, Niklas Loman, Håkan Olsson, Ulf Kristoffersson, Helena Jernström, Katja Harbst and Karin Henriksson, Lund University Hospital; Annika Lindblom, Brita Arver, Anna von Wachenfeldt, Annelie Liljegren, Gisela Barbany-Bustinza and Johanna Rantala, Stockholm, Karolinska University Hospital.

#### University of California Irvine (UCI)

SLN was partially supported by NIH CA74415 and the Morris and Horowitz Families Endowed Professorship.

#### University of California San Francisco (UCSF)

Grant funding from the NIH, NCI Bay Area Breast Cancer SPORE (P50 CA 058207) Grant funding from the Avon Foundation

Support from the UCSF Helen Diller Family Comprehensive Cancer Center

#### UK and Gilda Radner Familial Ovarian Cancer Registries (UKGRFOCR)

UKFOCR was supported by a project grant from CRUK to Paul Pharoah. We thank Paul Pharoah, Carole Pye, Patricia Harrington and Eva Wozniak for their contributions towards the UKFOCR. We'd like to acknowledge the Roswell Park Alliance Foundation for their continued support of the Gilda Radner Ovarian Family Cancer Registry. GRFOCR would like to acknowledge Kirsten Moysich (Department of Cancer Prevention and Control).

#### University of Pennsylvania (UPENN)

Breast Cancer Research Foundation (to KLN); Cancer Genetics Network (to SMD), Marjorie Cohen Foundation (to SMD)

#### Women's Cancer Research Institute (WCRI)

WCRI acknowledges support from the American Cancer Society, SIOP-06-258-01-CCE

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## 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.

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## 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

	<i>BRCAI</i> n (%) <sup>*</sup>	<i>BRCA2</i> n (%) <sup>*</sup>	OR∱	95%CI	OR∜	95%CI
Morphology						
invasive Ductal	2,387 (80)	1,515 (83)	1.00		ī	
invasive Lobular	67 (2.2)	153 (8.4)	3.3	(2.4-4.4)	ī	,
Medullary ${}^{S}$	281 (9.4)	40 (2.2)	0.25	(0.18-0.35)	ī	
Other	258 (8.6)	116 (6.4)	0.81	(0.63 - 1.02)	ı	·
ER, PR, HER2, TN						
<b>ER-positive</b>	625 (22)	1,475 (77)	11.4	9.8-13.2	10.0	8.2-12.1
R-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
Von-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

oercentage (%) 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 l tumors; analyses were adjusted for country and age at diagnosis

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

 $\mathcal{S}$  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

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BRCA1 mutation carriers   BRCA2 mutation carriers     Type   ER- negative n(%) <sup>*</sup> BRCA2 mutation carriers     Type   ER- n(%) <sup>*</sup> BRCA2 mutation carriers     Grade   In(%) <sup>*</sup> N(%)   N(%)   OR     Grade   In(%) <sup>*</sup> In(%)   N(%)   In(%)   N(%)     Grade   In(%)   In(%)   N(%)   In(%)   N(%)   In(%)     Grade   In(%)   In(%)   In(%)   In(%)   In(%)   In(%)     Grade   In(10.117) <thin(10.12< th=""><th></th><th></th><th></th><th></th><th></th><th></th><th></th></thin(10.12<>							
TypeER- negative $n (\%_0)^*$ ER- positive $n (\%_0)^*$ ER- positive $n (\%_0)^*$ ER- positive $n (\%_0)^*$ OR positive $n (\%_0)^*$ OR positive $n (\%_0)^*$ OR positive $n (\%_0)^*$ OR positive $n (\%_0)^*$ OR positive $n (\%_0)^*$ OR positive $n (\%_0)^*$ OR positive 		BRCA	I mutation c	arriers	BRC	A2 mutation	carriers
GradeGrade 1 $18 (1.2)$ $28 (6.9)$ $1.00$ $12 (4.3)$ $68 (7.4)$ $1.00$ Grade 2 $191 (13.1)$ $170 (41.6)$ $0.57 (0.31)$ $68 (7.4)$ $1.00$ Grade 2 $191 (13.1)$ $170 (41.6)$ $0.57 (0.31)$ $68 (7.4)$ $1.00$ Grade 2 $191 (13.1)$ $170 (41.6)$ $0.57 (0.31)$ $67 (24)$ $442 (48.2)$ $1.00$ Grade 3 $1246 (85.7)$ $210 (51.5)$ $0.12 (0.07)$ $200 (71.7)$ $408 (44.4)$ $0.33 (0.17-0.6)$ Morphology $0.21)$ $0.12 (0.07)$ $200 (71.7)$ $408 (44.4)$ $0.33 (0.17-0.6)$ Morphology $1.353 (58.8)$ $385 (86.5)$ $0.12 (0.07)$ $200 (71.7)$ $408 (44.4)$ $0.33 (0.17-0.6)$ Ductal $1.353 (58.8)$ $385 (86.5)$ $0.12 (0.07)$ $206 (85.8)$ $889 (84)$ $0.33 (0.17-0.6)$ Ductal $1.353 (58.8)$ $385 (86.5)$ $0.12 (0.07)$ $216 (85.8)$ $889 (84)$ $0.33 (0.17-0.6)$ Ductal $1.353 (58.8)$ $385 (86.5)$ $0.12 (10.7)$ $0.10 (10.4)$ $0.10 (10.4)$ Modullary 6 $117 (3.8)$ $0.11 (3.8)$ $0.11 (10.4)$ $0.11 (10.4)$ $0.11 (10.4)$ Other $91 (15)$ $22 (5)$ $0.12 (12)$ $13 (12)$ $0.11 (10.4)$	Type	ER- negative n (%)*	ER- positive n (%)	OR <sup>†</sup> (95%CI)	ER- negative n (%)	ER- positive n (%)	OR (95%CI)
Grade 1 $18(1.2)$ $28(6.9)$ $1.00$ $12(4.3)$ $68(7.4)$ $1.00$ Grade 2 $191(13.1)$ $170(41.6)$ $0.57(0.31)$ $67(24)$ $442(48.2)$ $1.2(0.60-2.4)$ Grade 3 $1246(85.7)$ $210(51.5)$ $0.12(0.07)$ $200(71.7)$ $408(44.4)$ $0.33(0.17-0.6)$ Morphology $1.353(58.8)$ $385(86.5)$ $0.12(0.07)$ $200(71.7)$ $408(44.4)$ $0.33(0.17-0.6)$ Morphology $1.353(58.8)$ $385(86.5)$ $-266(85.8)$ $889(84)$ $-$ Ductal $1,353(58.8)$ $385(86.5)$ $ 15(4.8)$ $110(10.4)$ $-$ Mothlary $\delta$ $141(23.5)$ $17(3.8)$ $ 14(4.6)$ $13(1.2)$ $-$ Other $91(15)$ $22(5)$ $ 15(4.8)$ $13(1.2)$ $-$	Grade						
Grade 2 $191 (13.1)$ $170 (41.6)$ $0.57 (0.31)$ $67 (24)$ $442 (48.2)$ $1.2 (0.60-2.4)$ Grade 3 $1246 (85.7)$ $210 (51.5)$ $0.12 (0.07)$ $200 (71.7)$ $408 (44.4)$ $0.33 (0.17-0.6)$ Morphology $0.21$ $0.21$ $0.21$ $200 (71.7)$ $408 (44.4)$ $0.33 (0.17-0.6)$ Morphology $1.353 (58.8)$ $385 (86.5)$ $0.21 (0.07)$ $206 (85.8)$ $889 (84)$ $-$ Ductal $1.353 (58.8)$ $385 (86.5)$ $ 266 (85.8)$ $889 (84)$ $-$ Modullary $\delta$ $14(123.5)$ $21 (4.7)$ $ 15 (4.8)$ $110 (10.4)$ $-$ Motullary $\delta$ $141 (23.5)$ $17 (3.8)$ $ 14 (4.6)$ $13 (1.2)$ $-$ Other $91(15)$ $22 (5)$ $ 15 (4.8)$ $46 (4.3)$ $-$	Grade 1	18 (1.2)	28 (6.9)	1.00	12 (4.3)	68 (7.4)	1.00
Grade 3 $1246 (85.7)$ $210 (51.5)$ $0.12 (0.07)$ $200 (71.7)$ $408 (44.4)$ $0.33 (0.17-0.6)$ Morphology $0.21)$ $0.21)$ $2.00 (71.7)$ $408 (44.4)$ $0.33 (0.17-0.6)$ Morphology $1.353 (58.8)$ $385 (86.5)$ $2.06 (85.8)$ $889 (84)$ $-$ Ductal $1.353 (58.8)$ $385 (86.5)$ $ 266 (85.8)$ $889 (84)$ $-$ Morphology $16(2.7)$ $21 (4.7)$ $ 15 (4.8)$ $110 (10.4)$ $-$ Medullary $\delta$ $141 (23.5)$ $17 (3.8)$ $ 14 (4.6)$ $13 (1.2)$ $-$ Other $91 (15)$ $22 (5)$ $ 15 (4.8)$ $46 (4.3)$ $-$	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)
Morphology 266 (85.8) 385 (86.5) 266 (85.8) 889 (84) -   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) -	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)
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)   -	Morphology						
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)$ $46(4.3)$ $-$	Ductal	1,353 (58.8)	385(86.5)		266 (85.8)	889 (84)	
Medullary §   141(23.5)   17 (3.8)   -   14 (4.6)   13 (1.2)   -     Other   91(15)   22 (5)   -   15 (4.8)   46 (4.3)   -	Lobular	16(2.7)	21 (4.7)		15 (4.8)	110 (10.4)	
<b>Other</b> 91(15) 22 (5) - 15 (4.8) 46 (4.3) -	Medullary $^{\&}$	141(23.5)	17 (3.8)	I	14 (4.6)	13 (1.2)	ı
	Other	91(15)	22 (5)	ı	15 (4.8)	46 (4.3)	ı
	$^{\dagger}$ OR and 95%Cl country of origin	I are for ER-poon	sitive vs. ER-1	regative diseas	e in Grade 2 o.	r Grade 3 tum	ors are compared
$\overset{7}{/}$ OR and 95%CI are for ER-positive vs. ER-negative disease in Grade 2 or Grade 3 tumors are compared with country of origin.	$\hat{s}_{\text{includes medul}}$	llary and atypic	al medullary t	umours.			

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		<i>BRCA1</i> n (%)	<i>BRCA2</i> 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