Association of Type and Location of BRCA1 and BRCA2 Mutations With Risk of Breast and Ovarian Cancer

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IMPORTANTANCE Limited information about the relationship between specific mutations in BRCA1 or BRCA2 (BRCA1/2) and cancer risk exists.

OBJECTIVE To identify mutation-specific cancer risks for carriers of BRCA1/2.

DESIGN, SETTING, AND PARTICIPANTS Observational study of women who were ascertained between 1937 and 2011 (median, 1999) and found to carry disease-associated BRCA1 or BRCA2 mutations. The international sample comprised 19,581 carriers of BRCA1 mutations and 11,900 carriers of BRCA2 mutations from 55 centers in 33 countries on 6 continents. We estimated hazard ratios for breast and ovarian cancer based on mutation type, function, and nucleotide position. We also estimated RHR, the ratio of breast vs ovarian cancer hazard ratios. A value of RHR greater than 1 indicated elevated breast cancer risk; a value of RHR less than 1 indicated elevated ovarian cancer risk.

EXPOSURES Mutations of BRCA1 or BRCA2.

MAIN OUTCOMES AND MEASURES Breast and ovarian cancer risks.

RESULTS Among BRCA1 mutation carriers, 9,052 women (46%) were diagnosed with breast cancer, 2,317 (12%) with ovarian cancer, 10,41 (5%) with breast and ovarian cancer, and 7,717 (37%) without cancer. Among BRCA2 mutation carriers, 6,180 women (52%) were diagnosed with breast cancer, 682 (6%) with ovarian cancer, 272 (2%) with breast and ovarian cancer, and 4,766 (40%) without cancer. In BRCA1, we identified 3 breast cancer cluster regions (BCCRs) located at c.179 to c.505 (BCCR1; RHR = 1.46; 95% CI, 1.22-1.74; P = 2 × 10⁻⁴⁹), c.4328 to c.4945 (BCCR2; RHR = 1.34; 95% CI, 1.01-1.78; P = .04), and c.5261 to c.5563 (BCCR2′; RHR = 1.38; 95% CI, 1.22-1.55; P = 6 × 10⁻⁸⁸). We also identified an ovarian cancer cluster region (OCCR) from c.1380 to c.4062 (approximately exon 11) with RHR = 0.62 (95% CI, 0.56-0.70; P = 9 × 10⁻¹⁰). In BRCA2, we observed multiple BCCRs spanning c.1 to c.596 (BCCR1; RHR = 1.71; 95% CI, 1.06-2.78; P = .03), c.772 to c.1806 (BCCR1′; RHR = 1.63; 95% CI, 1.10-2.40; P = .01), and c.7394 to c.8904 (BCCR2; RHR = 2.31; 95% CI, 1.69-3.16; P = .00002). We also identified 3 OCCRs: the first (OCCR1) spanned c.3249 to c.5681 that was adjacent to c.5946delT (6174delC7; RHR = 0.51; 95% CI, 0.44-0.60; P = 6 × 10⁻¹⁵). The second OCCR spanned c.6645 to c.7471 (OCCR2; RHR = 0.57; 95% CI, 0.41-0.80; P = .001). Mutations conferring nonsense-mediated decay were associated with differential breast or ovarian cancer risks and an earlier age of breast cancer diagnosis for both BRCA1 and BRCA2 mutation carriers.

CONCLUSIONS AND RELEVANCE Breast and ovarian cancer risks varied by type and location of BRCA1/2 mutations. With appropriate validation, these data may have implications for risk assessment and cancer prevention decision making for carriers of BRCA1 and BRCA2 mutations.

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Women who have inherited mutations in BRCA1 (17q21, chromosome 17; base pairs 43,044,294 to 43,125,482) or BRCA2 (13q12.3, chromosome 13; base pairs 32,315,479 to 32,399,671) have an increased risk of breast and ovarian cancers.\(^1^2\) Little is known about how cancer risks differ by BRCA1 or BRCA2 (BRCA1/2) mutation type. An “ovarian cancer cluster region” (OCCR) has been reported in both BRCA1 and BRCA2 using small sample sets. For BRCA1, initially mutations after exon 11 were associated with a 20% lower ovarian cancer risk than mutations in exons 1 through 11.\(^3\) Following that observation, Thompson et al\(^4\) reported an increased risk of ovarian vs breast cancer specifically was associated with mutations in the central portion of exon 11. This association was attributed to both a decrease in breast cancer risk and an increase in ovarian cancer risk in this region. Mutations in exon 11 of BRCA2 also have been associated with higher ovarian vs breast cancer risk than in other regions of the gene.\(^5\) It was hypothesized that this risk variation might be explained by the failure of BRCA1/2 exon 11 truncating mutations to trigger nonsense-mediated messenger RNA (mRNA) decay (NMD) because of their extremely large size, contrary to truncating mutations in smaller exons. However, this postulate was not supported by the measures of the relative amounts of mRNA transcript encoded by BRCA1/2 alleles.\(^6^7\)

Murine models of different mutations in BRCA1/2 also suggest that genotype-phenotype correlations exist.\(^8^9\) To our knowledge, no study has reported whether BRCA1/2 mutation type is associated with differences in breast and ovarian cancer risk. Thus, we evaluated whether BRCA1 and BRCA2 mutation type or location is associated with variation in breast and ovarian cancer risk.

Methods

The Consortium of Investigators of Modifiers of BRCA (CIMBA) initiative is an international collaboration of centers on 6 continents that has collected information about carriers of disease-associated BRCA1 and BRCA2 mutations with associated clinical, risk factor, and genetic data.\(^10\) All carriers participated in clinical assessment or research studies at the host institutions after providing informed consent under protocols approved by institutional review boards. For some individuals, ascertainment date reflects the earliest date at which they came to the attention of a clinician or research investigator (eg, when they were first seen in a clinic), even though their research participation, genetic testing, and research data collection may have occurred many years later. Fifty-five centers and multicenter consortia (eTable 1 in the Supplement) in 33 countries submitted deidentified data that met the CIMBA inclusion criteria.\(^10\) Study eligibility criteria included carriage of a disease-associated mutation and clinical data necessary to estimate hazard ratios (ie, cancer diagnosis, ascertainment and follow-up dates). Women were excluded if they carried both a BRCA1 and BRCA2 mutation (n = 84).

No races/ethnicities were excluded from this study. All races/ethnicities were included in this report to provide maximal generalizability of results for populations who may be undergoing genetic testing and counseling. All race/ethnicity designations were based on self-report. Race/ethnicity data were collected across the various centers using either fixed categories or open-ended questions.

Mutation Classification

Only carriers with clearly pathogenic BRCA1/2 mutations were included in this analysis. Pathogenic mutations were defined as (1) mutations generating a premature termination codon, except variants generating a premature termination codon in exon 27 after codon 3010 of BRCA2\(^2^3\); (2) large in-frame deletions that span 1 or more exons; and (3) deletions of transcription regulatory regions (promoter and/or first exon) expected to cause lack of expression of mutant allele. We also included missense variants considered pathogenic by the Breast Cancer Information Core committee or published variants classified as pathogenic using multifactorial likelihood approaches.\(^12^13\)

Mutations are described here using the Human Genome Variation Society nomenclature in which the nucleotide numbering is from the A of the ATG translation initiator codon, and use the c.XXX numbering convention (eAppendix 1 in the Supplement).

Creation of Mutation Groups for Analysis

Mutation Bins

To identify segments across the intronic and exonic regions of the BRCA1 or BRCA2 genes associated with different breast vs ovarian cancer risks, we created bins of mutations by base pair location (Figure 1). We divided the genomic regions of both genes to create bins of genomic sequence that contained...
all deleterious mutations regardless of category or function. Bins were constructed by using an algorithm in which each bin contained approximately equal numbers of participants with bin length defined by distance in base pairs. We excluded large genomic rearrangements from this analysis as those mutations span multiple bins and also undertook a subset analysis with and without missense mutations. The resulting bins are presented in Figure 2 and eTable 2 in the Supplement for BRCA1 and Figure 3 and eTable 3 in the Supplement for BRCA2.

Mutation Type and Functional Domains
Mutations were grouped by type and function as frame shift, nonsense, missense, splice site, and then by in-frame and out-of-frame. Mutation groups included individuals who carried in-frame deletions, nonsplice out-of-frame deletions, and out-of-frame deletions. Missense mutations in BRCA1 were grouped into those within the RING14,15 and BRCT domains.16-19 Only 17 BRCA2 carriers (0.1%) had missense mutations classified as pathogenic; these were removed from the analysis because the sample size was too small to provide statistically meaningful inferences. Comparisons also were made of mutations predicted not to lead to NMD vs those that do lead to NMD. Mutations predicted not to cause NMD were defined as those that lead to a stop codon within 50 nucleotides before or within the last exon.20 In BRCA1, a subgroup including premature termination codons before c.297, presumed to allow reinitiation of translation at the AUG at that site, was examined separately.21 Premature termination codons refer to all mutations leading

Figure 2. Hazard Ratio of Breast Cancer Relative to the Hazard Ratio of Ovarian Cancer by BRCA1 Nucleotide Position

The graph shows the ratio of hazard ratios (blue data markers) and 95% CI (error bars) for the mutation bins defined across the span of the coding DNA sequence of the BRCA1 gene. Black arrowheads under the bins indicate 2 founder mutations of clinical interest in the Ashkenazi Jewish population. Regions inferred to be breast cancer cluster regions (BCCRs) and ovarian cancer cluster regions (OCCRs) are shown at the bottom. Solid light blue lines indicate regions found to be statistically significant; dashed light blue lines indicate regions in the same direction of effect that were not statistically significant. eTable 2 in the Supplement lists the bins and risks used to define the BCCRs and OCCRs.
to a truncated open reading frame. Putative functional domains in BRCA1 and BRCA2 were defined using the boundaries in the Pfam database.\textsuperscript{22} We also identified reported domains in BRCA1 or BRCA2 that are involved in binding putative proteins.

**Statistical Analysis**

The primary outcomes of interest were diagnosis of ovarian cancer or breast cancer. For ovarian cancer, observations were censored at the earliest of the following outcomes: bilateral risk-reducing salpingo-oophorectomy, death, or having reached the end of follow-up without an ovarian cancer or other censoring event. In women with both breast and ovarian cancer diagnoses, prior breast cancer diagnoses were ignored in the analysis of ovarian cancer. Time to event was computed from birth to age at first ovarian cancer diagnosis or age at censoring. For the primary event of breast cancer, observations were censored at the earliest of the following outcomes: ovarian cancer, risk-reducing salpingo-oophorectomy, risk-reducing mastectomy, death, or having reached the end of follow-up without a breast cancer or other censoring event. Time to event was computed from birth to age at first cancer diagnosis or age at...

**Figure 3. Hazard Ratio of Breast Cancer Relative to the Hazard Ratio of Ovarian Cancer by BRCA2 Nucleotide Position**

The graph shows the ratio of hazard ratios (blue data markers) and 95% CI (error bars) for the mutation bins defined across the span of the coding DNA sequence of the BRCA2 gene. The black arrowhead under the bins indicates a foundermutation of clinical interest in the Ashkenazi Jewish population. The regions inferred to be breast cancer cluster regions (BCCRs) and ovarian cancer cluster regions (OCCRs) are shown at the bottom; the solid light blue lines indicate regions found to be statistically significant. eTable 3 in the Supplement lists the bins and risks used to define the BCCRs and OCCRs.
censoring. To account for intracluster dependence due to multiple individuals from the same family, a robust sandwich variance estimate was specified in Cox proportional hazards models. All analyses were undertaken in BRCA1 and BRCA2 mutation carriers separately. The proportional hazards assumption was tested using \( log(-log) \) plots and Schoenfeld residuals.

Our analyses assessed the relationship of mutation groups with cancer risk. First, we used mutation bins to evaluate whether there is evidence to support the previous report of an OCCR and whether breast cancer cluster regions (BCCRs) may exist. To assess whether specific genomic regions of these genes were associated with greater breast vs ovarian cancer risk, we computed the hazard ratio of breast cancer, the hazard ratio of ovarian cancer, and a statistic \( \text{RHR} \) defined as the ratio of breast vs ovarian cancer hazard ratio estimates. Values of \( \text{RHR} \) greater than 1 indicate elevated breast cancer risk; values of \( \text{RHR} \) less than 1 indicate elevated ovarian cancer risk. We evaluated bins of mutations across the span of BRCA1 or BRCA2 compared with all other mutations not contained in that bin by fitting a multiple correlated outcomes model stratified by cancer site. This approach allowed us to achieve 2 goals: first, to estimate the correlation between ovarian and breast cancer outcomes within an individual, and second, to provide an estimate of the \( \text{RHR} \) (estimated via an interaction term between cancer site and mutation bin) with the correct confidence interval using robust sandwich variance estimates to account for the correlation between outcomes within a woman. All analyses were adjusted for birth year and race, stratified by center, and controlled for clustering within family.

Second, we compared each mutation type or functional group against a common reference group. The use of a common reference group allowed us to compare hazard ratio estimates across different mutation classes. For both BRCA1 and BRCA2, we chose exon 11 nonsense mutations as the common reference group. Exon 11 nonsense mutations are common in diverse ethnic backgrounds and have been demonstrated to have the same biological effect, leading to NMD.

Approximate cancer risks to age 70 years for specific mutation classes were derived from the relative risk estimates. For BRCA1 and BRCA2, estimated lifetime breast cancer penetrances were assumed to be 59% and 51% and ovarian cancer penetrance 34% and 11%, respectively. Mutation-specific penetrance estimates were derived using the method presented in eAppendix 2 in the Supplement.

Statistical tests were judged significant based on 2-sided hypothesis tests with \( P < .05 \). All \( P \) values were corrected for multiple hypothesis testing within each table of results by controlling the false discovery rate (FDR) using the method of Benjamini and Hochberg. Analyses were conducted in SAS version 9 (SAS Institute) or R version 2.7.2 (R Foundation for Statistical Computing).

**Results**

A total of 19,581 female carriers of BRCA1 mutations and 11,900 carriers of BRCA2 mutations were eligible for inclusion in this study. Table 1 reports the distribution of dates of ascertainment to the study as well as time from ascertainment to cancer diagnosis or censoring, as used in the survival analysis models reported in this section. Mean age of breast cancer diagnosis was 39.9 years in BRCA1 mutation carriers and 42.8 years in BRCA2 mutation carriers. Mean age of ovarian cancer diagnosis was 50.0 years in BRCA1 mutation carriers and 54.5 years in BRCA2 mutation carriers. The 3 ovarian cancer cases diagnosed before age 18 years were germ cell tumors and included in the analysis (Table 1). Of note, all analyses also were undertaken excluding these 3 cases and there was no difference in the results. The majority of the sample consisted of white women for both BRCA1 and BRCA2 mutation carriers: 92% to 93% white, including 8% to 9% Jewish women. Both BRCA1 and BRCA2 mutation carriers had a median parity of 2.0 live births and age at menarche of 13 years. Median age at menopause was 44 years in BRCA1 and 46 years in BRCA2 mutation carriers, reflecting in part the use of preventive surgeries.

**BRCA1: Breast and Ovarian Cancer Cluster Regions**

We observed an OCCR bounded by c.1380 and c.4062 (Figure 2), suggesting a relative decrease in breast relative to ovarian cancer risk (\( \text{RHR} = 0.62; \) 95% CI, 0.56-0.70; FDR-corrected \( P = 9 \times 10^{-7} \)). This estimate was obtained by considering all mutations across multiple bins spanning the OCCR. The OCCR is explained by both a relative decrease in breast cancer risk and a relative increase in ovarian cancer risk (eTable 2 in the Supplement), which was statistically significant in bins 9, 11-13, 15-16, and 23 (Figure 2). The OCCR extends further \( 5 ' \) of the previously reported OCCR, which was defined by the interval c.2282 to c.4071.3 The OCCR is entirely contained within exon 11 (c.670-c.4096) with bins 6 and 23 being approximately coincident with the boundaries of the exon.

We also observed a relative increase in breast cancer risk and a relative decrease in ovarian cancer risk for mutations occurring in the \( 5 ' \) and \( 3 ' \) regions of BRCA1, potentially defining 2 BCCRs (Figure 2). BCCR1 mutations within bins 4-5 (c.179-c.505) were associated with excess risks of breast vs ovarian cancer (eTable 2 in the Supplement) and lie within the \( 3 ' \) region of the RING domain (c.72-c.192). Mutations in the BCCR1 were associated with a relative increase in breast cancer risk relative to ovarian cancer risk (\( \text{RHR} = 1.46; \) 95% CI, 1.22-1.74; FDR-corrected \( P = 2 \times 10^{-6} \)). When all mutations in the RING domain were considered together as compared with all others, they were associated with a significant increase in breast cancer risk (HR = 1.13; 95% CI, 1.02-1.26) and a significant decrease in ovarian cancer risk (HR = 0.81; 95% CI, 0.67-0.97). Bin 2, which contains only the founder mutation BRCA1 c.68_69delAG (185delAG), did not provide statistically significant evidence for elevated breast vs ovarian cancer risks, suggesting that this mutation is associated with relatively equivalent risks of both cancers.

Mutations in bins 26 and 29-30 in the \( 3 ' \) region of BRCA1 also provided evidence for additional BCCRs. BCCR2 was associated with an increase in breast cancer relative to ovarian cancer risk (\( \text{RHR} = 1.34; \) 95% CI, 1.01-1.78; \( P = .04 \)) bounded by c.4328 and c.4945. The second segment of this BCCR (denoted BCCR2') includes the BRCT domains (c.4926-c.5169 and
c.5268-c.5526) and was associated with a relative excess of breast vs ovarian cancers (RHR = 1.38; 95% CI, 1.22-1.55; \( P = 6 \times 10^{-9} \)) (Figure 2). In the BRCT domains, the preponderance of mutations was missense, not expected to trigger NMD. This region also includes bin 29, which contains only the BRCA1 c.5266dupC (5382insC) mutation, which also is not predicted to lead to NMD as it introduces a premature termination codon in the last exon.6 In bin 30, BRCA1 c.5277 + 1G>A, a common splice site mutation in the Netherlands, is observed and not expected to lead to NMD.

We compared breast and ovarian cancer risks between women who had a mutation in a specified functional domain compared with all other women who did not have mutations in that domain. Mutations in the RING domain were associated with higher breast cancer risks and nonsignificant lower ovarian cancer risks than other mutations. These results are consistent with the colocation of the BCCR1 (Figure 2) and RING domain. Mutations in the BRCT domains were associated with higher breast cancer risk. When analyses were limited to mutations conferring NMD, breast cancer risk became significantly associated with mutations in the coiled coil domain.

**BRCA1: Risks by Category and Function**

We observed variability in breast and ovarian cancer risks by mutation class (Table 2 and Table 3). For BRCA1-associated breast cancer, most risk groups were associated with higher breast cancer risk than the exon 11 nonsense mutation reference group. This result is consistent with the data shown in Figure 2 and eTable 2 in the Supplement as it is similar in location with the OCCR. Groups with elevated breast cancer risk include all mutations leading to NMD (group 1), all premature termination codon mutations except for exon 11 nonsense mutations (group 2), frame shift and nonsense mutations occurring 5′ of c.297 that are predicted to lead to NMD and reinitiation (group 3), nonpremature termination codon mutations (group 4), all founder mutations (group 5), and the founder mutation c.5266dupC (group 5b), missense mutations (group 6, 6a), missense and in-frame deletions (group 7), all in-frame deletions (group 8), and premature termination codon mutations not leading to NMD (group 9). The majority of the last group is comprised of c.5266dupC (83%). For BRCA1-associated ovarian cancer (Table 2), mutations associated with significantly lower ovarian cancer risks compared with the reference group included mutations 5′ of c.297 (group 3), nonpremature termination codons (group 4), founder mutations (group 5, 5a, 5b), missense mutations (group 6, 6a), missense and in-frame deletions (group 7), and premature termination codons not leading to NMD (group 9).

When comparing mean age differences among women with or without a specific mutation category or function, we found small but statistically significant differences. In BRCA1, exon 11 mutations were associated with earlier ages at breast and ovarian cancer diagnosis. Mutations conferring NMD or premature termination codon were associated with a later age at breast cancer diagnosis. Conversely, an earlier

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Table 1. Characteristics of Study Sample: Ascertainment, Diagnosis, Demographics, and Risk Factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>BRCA1 Mutation Carriers</th>
<th>BRCA2 Mutation Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Median or Mean (Range)</td>
</tr>
<tr>
<td>Women with breast cancer</td>
<td>10,093</td>
<td>6452</td>
</tr>
<tr>
<td>Mean age at breast cancer diagnosis, y</td>
<td>39.9 (17-85)</td>
<td>9.2</td>
</tr>
<tr>
<td>Women without breast cancer</td>
<td>9,488</td>
<td>5,448</td>
</tr>
<tr>
<td>Mean age of women with no breast cancer diagnosis, y</td>
<td>41.0 (12-102)</td>
<td>12.0</td>
</tr>
<tr>
<td>Women with ovarian cancer</td>
<td>3,358</td>
<td>954</td>
</tr>
<tr>
<td>Mean age at ovarian cancer diagnosis, y</td>
<td>50.0 (16-92)</td>
<td>9.5</td>
</tr>
<tr>
<td>Women without ovarian cancer</td>
<td>16,223</td>
<td>10,946</td>
</tr>
<tr>
<td>Mean age of women with no ovarian cancer diagnosis, y</td>
<td>42.0 (12-102)</td>
<td>12.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race/ethnicity</th>
<th>BRCA1 Mutation Carriers</th>
<th>BRCA2 Mutation Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>16,481</td>
<td>10,014</td>
</tr>
<tr>
<td>African/African American</td>
<td>176</td>
<td>87</td>
</tr>
<tr>
<td>Asian</td>
<td>392</td>
<td>404</td>
</tr>
<tr>
<td>Hispanic</td>
<td>333</td>
<td>175</td>
</tr>
<tr>
<td>Jewish</td>
<td>1,800</td>
<td>971</td>
</tr>
<tr>
<td>Other</td>
<td>399</td>
<td>249</td>
</tr>
<tr>
<td>Parity, No. of live births</td>
<td>2.0 (0-14)</td>
<td>1.4</td>
</tr>
<tr>
<td>Age at menarche, y</td>
<td>13.0 (8-23)</td>
<td>1.5</td>
</tr>
<tr>
<td>Age at natural or surgical menopause, y</td>
<td>44.0 (16-68)</td>
<td>6.3</td>
</tr>
</tbody>
</table>

*Includes age at time of original family ascertainment for some women who were never diagnosed with cancer.
*bIncludes 3 germ cell carcinoma cases diagnosed before age 21 years.
age at breast cancer diagnosis was associated with nonpre-
mature termination codon mutations and the founder
mutations (Table 3).

**BRCA2: Breast and Ovarian Cancer Cluster Regions**

We observed an OCCR (OCCR1) bounded by c.3249 and
c.5681, containing c.5946delT (6174delT), with statistically
significant evidence for a relatively higher ovarian cancer vs
breast cancer risk among carriers of mutations in bins 6-9
and 11 (Figure 3). OCCR1 is explained by both a relative
increase in ovarian cancer risk and a relative decrease in
breast cancer risk, with an increase in ovarian cancer rela-
tive to breast cancer risk (RHR = 0.51; 95% CI, 0.44-0.60;
P = 6 × 10^{-17}) (eTable 3 in the Supplement). The putative
OCCR1 lies within the previously reported OCCR3 and
approximately colocalized with the BRC repeats within exon
11.27,18,28 A second putative OCCR (OCCR2) outside of
the original OCCR boundaries also was observed defined by bin
14 (c.6645-c.7471). OCCR2 was associated with an increase in ovarian cancer relative to breast cancer risk (RHR = 0.57; 95% CI, 0.41-0.80; P = .001).

We also observed a relative increase in breast cancer risk and a relative decrease in ovarian cancer risk for mutations occurring in the 5′ and 3′ regions of \(\text{BRCA2}\), potentially defining multiple BCCRs (ie, BCCR1, BCCR1′, and BCCR2) (Figure 3). These 3 regions were associated with relatively increased breast cancer risk relative to ovarian cancer risk with RHR = 1.71 (95% CI, 1.06-2.78; P = .03), RHR = 1.63 (95% CI, 1.10-2.40; P = .01), and RHR = 2.31 (95% CI, 1.69-3.16; P = .00002), respectively.

These regions were associated with both increased breast cancer risk and decreased ovarian cancer risk (eTable 3 in the Supplement).

We also observed small but statistically significant differences in the mean age at breast cancer diagnosis associated with some of these regions. The mean age was greater for mutations in OCCR vs mutations not in OCCR (45.0 vs 43.9 years, P < .001; mean difference: 1.17; 95% CI, 0.65 to 1.69), lower for mutations in BCCR1 vs mutations not in BCCR1 (42.4 vs 44.3 years; P = .004; mean difference: −1.66; 95% CI, −2.80 to −0.53), and lower for mutations in BCCR2 vs mutations not in BCCR2 (43.5 vs 44.3 years, P = .04; mean difference: −0.80, 95% CI, −1.55 to −0.05).

To complement the prior set of analyses, we also present associations of breast and ovarian cancers among groups of \(\text{BRCA2}\) mutation carriers defined by known DNA binding domains (Table 4). Mutations in the BRC repeats were associated with lower breast cancer risks and higher ovarian cancer risks than those mutations not occurring in the BRC repeats consistent with their colocation with the OCCR1 (Figure 3).

### Table 3. Mutation-Specific Risk Groups: Ages at Diagnosis of Breast Cancer or Ovarian Cancer

<table>
<thead>
<tr>
<th>Group*</th>
<th>No. of Women With Breast Cancer</th>
<th>Mean Age at Diagnosis, y</th>
<th>Breast Cancer</th>
<th>Ovarian Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With Mutation</td>
<td>Without Mutation</td>
<td>FDR P Value</td>
<td>With Mutation</td>
</tr>
<tr>
<td>BRCA1 (n = 19 581)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>796</td>
<td>336</td>
<td>40.4</td>
<td>42.4</td>
</tr>
<tr>
<td>1</td>
<td>5469</td>
<td>2038</td>
<td>41.2</td>
<td>40.1</td>
</tr>
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<td>2524</td>
<td>41.2</td>
<td>40.4</td>
</tr>
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<td>3</td>
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<td>74</td>
<td>40.7</td>
<td>39.4</td>
</tr>
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<td>4</td>
<td>2986</td>
<td>781</td>
<td>40.5</td>
<td>41.1</td>
</tr>
<tr>
<td>5</td>
<td>2698</td>
<td>850</td>
<td>40.2</td>
<td>41.1</td>
</tr>
<tr>
<td>5a</td>
<td>1033</td>
<td>391</td>
<td>40.43</td>
<td>42.41</td>
</tr>
<tr>
<td>5b</td>
<td>1665</td>
<td>459</td>
<td>40.5</td>
<td>41.5</td>
</tr>
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<td>6</td>
<td>899</td>
<td>241</td>
<td>40.6</td>
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<td>202</td>
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<td>7</td>
<td>925</td>
<td>249</td>
<td>40.6</td>
<td>40.9</td>
</tr>
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<td>155</td>
<td>43.33</td>
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Abbreviations: FDR, false discovery rate.
* Group descriptions appear in Table 2.
they do not account for noncancer outcomes that may influence a woman’s life expectancy, the effects of family history, and nonrandom ascertainment of mutation carriers in this sample and depend on assumptions about the prevalence of different mutation classes in the population. Using the \(\text{BRCA1/2}\) baseline breast and ovarian cancer risks of Antoniou et al,\(^{26}\) we estimated risks and confidence intervals about these risks (eAppendix 2 in the Supplement).

These confidence limits assume that the overall risk for a given individual is provided by our estimates and should not be interpreted as measuring the overall uncertainty in the absolute risk estimates, as shown in Table 5. The overall breast cancer risk for \(\text{BRCA1}\) mutation carriers by age 70 years is 59%, which increases to 69% (95% CI, 56%-83%) in women who carry a missense mutation, Jewish founder mutation, or a mutation that undergoes NMD with reinitiation. The ovarian cancer risk in \(\text{BRCA1}\) mutation carriers by age 70 years is 34% overall but decreases to 26% (95% CI, 10%-43%) among women who carry a founder mutation.

### Discussion

We have identified mutations in \(\text{BRCA1}\) or \(\text{BRCA2}\) that are associated with significantly different risks of breast and ovarian cancers. These mutation-specific risks coincide with known or hypothesized functional domains and provide a basis around which accurate risk estimates can be generated for women who have inherited a particular \(\text{BRCA1/2}\) mutation. These results are consistent with prior reports of OCCRs in both \(\text{BRCA1}\) and \(\text{BRCA2}\) that lie in or near exon 11 of both genes.\(^{3,5,29,30}\) Mutations in exon 11
could produce a partial BRCA1 protein encoded by the known exon 11 splice variant, while the full-length protein is lost by the process of NMD. Murine embryos carrying the exon 11-deleted isoform survive longer than those that are BRCA1 null, and BRCA1 that has lost exon 11 appear to retain partial function. Thus, for BRCA1, it is biologically plausible that individuals carrying mutations within exon 11 (and the OCCR) may have a different phenotype than other mutations. In BRCA2, we have identified OCCRs, coincident with the 8 BRC repeats. Mutations in this region appear to be associated with NMD, which would lead to loss of BRCA2 expression. However, it is possible that there is persistence of an alternatively spliced variant of BRCA2, without exon 11 (as for BRCA1), which would represent in-frame mutations. In addition, the BRCA2 BRC repeats interact with RAD51, which has been consistently shown to be a modifier of BRCA2-associated breast and ovarian cancer risk. Without the BRC repeats, BRCA2 might differ in interactions with RAD51 and lead to genotype-phenotype variation. However, the biological basis of the BRCA2 OCCR remains speculative, in particular as it does not extend throughout all of exon 11.

In BRCA2, several putative BCCRs were defined. The 3′ BCCR coincides approximately with mutations occurring in the oligonucleotide binding (OB) fold domains and the tower domain. When examined independently, both of these domains were associated with relatively elevated breast cancer risk and lower ovarian cancer risk. These mutations in BRCA2 would be predicted to undergo NMD. However, it has been demonstrated experimentally for only a few mutations, leaving the functional basis unknown.

We have also identified a decreased risk of ovarian cancer associated with all types of mutations predicted not to lead to NMD in BRCA2; the estimated risk was only significant for all mutations together and those mutations leading to in-frame splice site or frame shift mutations. These mutations all occur after nucleotide 7000 in the C-terminus of BRCA2, which includes the DNA binding domains, tower domains, and OB folds. These functional domains are associated with localization of BRCA2 to sites of double-stranded DNA breaks to accomplish repair. These data suggest that intact protein may be protective when it comes to ovarian cancer risk. However, the number of individuals is small and further replication is needed.

A number of limitations of this research may influence the generalizability and translational potential of this research. Despite the very large sample size, we were not able to investigate some mutation and risk groups with adequate statistical power. Carriers of BRCA2 mutations composed a smaller sample set; in particular, the number of women with BRCA2-associated ovarian cancers was relatively small. Although all women with a documented disease-associated mutation in the CIMBA database were included, some populations use screening for founder mutations as a primary method of mutation detection, such as for the 3 Ashkenazi Jewish mutations. This testing strategy may lead to underreporting of nonfounder mutations. As such, some bias in the ascertainment of the full spectrum of mutations could have occurred. The ascertainment strategy generally followed clinical and research protocols similar across all centers. However, we did not correct for ascertainment, and thus bias may have affected some variables (eg, age at diagnosis), which should be interpreted with caution. Mutation testing was performed using methods acceptable for clinical practice at each center, which was not uniform across all centers.

The present sample set does not reflect the general population of all mutation carriers but reflects those women who have undergone genetic testing for BRCA1/2 mutations, a relevant population of inference. We have presented the mutations in terms of category or effect, but these designations are in some cases extrapolated based on experimental evidence for similar mutations. An example is the designation of NMD inferred from mutation location, which is based on experimental validation of only a small proportion of the mutations. Similarly, inference of protein truncation based on predicted protein-truncating mutations without experimental verification may lead to erroneous classification. Penetrances that are presented here are limited because other factors that are not accounted for here could influence these estimates. These factors include family history and competing mortality. These risks also depend on knowing the true prevalence of the mutation-specific classes, which is likely to be population-specific.

In addition, the present report of more than 32 000 mutation carriers could include some of those individuals who were included in the 1995 and 1997 articles that originally reported the OCCR. It is not possible at this time to know if any of the 32 families carrying BRCA1 mutations or 25 families carrying BRCA2 originally reported also are included in the present sample. However, it is highly unlikely that the small sample of individuals represented in the original reports would outweigh a potential null effect among the more than 32 000 individuals studied here.

This study is the first step in defining differences in risk associated with location and type of BRCA1 and BRCA2 mutations. Pending additional mechanistic insights into the observed associations, knowledge of mutation-specific risks could provide important information for clinical risk assessment among BRCA1/2 mutation carriers, but further systematic studies will be required to determine the absolute cancer risks associated with different mutations. It is yet to be determined what level of absolute risk change will influence decision making among carriers of BRCA1/2 mutations. Additional research will be required to better understand what level of risk difference will change decision making and standards of care, such as preventive surgery, for carriers of BRCA1 and BRCA2 mutations.

Conclusions
Breast and ovarian cancer risks varied by type and location of BRCA1/2 mutations. With appropriate validation, these data may have implications for risk assessment and cancer prevention decision making among carriers of BRCA1 and BRCA2 mutations.


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