Candidate genetic modifiers for breast and ovarian cancer risk in BRCA1 and BRCA2 mutation carriers

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Abstract

**Background**—BRCA1 and BRCA2 mutation carriers are at substantially increased risk for developing breast and ovarian cancer. The incomplete penetrance coupled with the variable age at diagnosis in carriers of the same mutation suggests the existence of genetic and non-genetic modifying factors. In this study we evaluated the putative role of variants in many candidate modifier genes.

**Methods**—Genotyping data from 15,252 BRCA1 and 8,211 BRCA2 mutation carriers, for known variants (n=3,248) located within or around 445 candidate genes, were available through the iCOGS custom-designed array. Breast and ovarian cancer association analysis was performed within a retrospective cohort approach.

**Results**—The observed p-values of association ranged between 0.005–1.000. None of the variants was significantly associated with breast or ovarian cancer risk in either BRCA1 or BRCA2 mutation carriers, after multiple testing adjustments.

**Conclusion**—There is little evidence that any of the evaluated candidate variants act as modifiers of breast and/or ovarian cancer risk in BRCA1 or BRCA2 mutation carriers.

**Impact**—Genome-wide association studies have been more successful at identifying genetic modifiers of BRCA1/2 penetrance than candidate gene studies.

**Keywords**

BRCA1 BRCA2 mutations; BRCA-mutation carriers; Breast cancer risk; Ovarian cancer risk; Candidate genetic risk modifiers

**Introduction**

Germline BRCA1 or BRCA2 mutations substantially increase the risk of developing breast and ovarian cancer over those of the general population (1). The penetrance is incomplete and combined with the observed variability in age at cancer diagnosis in carriers of identical mutations, suggests the existence of genetic and/or environmental modifying factors. Direct evidence for genetic modifiers of breast and ovarian cancer risk for BRCA1 and BRCA2...
mutation carriers has been provided through genome-wide association studies (GWAS) (2). In parallel, multiple variants in candidate genes that affect BRCA1 or BRCA2 protein expression, act along the same biological pathways, or physically interact with BRCA1 or BRCA2 proteins have been evaluated as putative modifiers of BRCA1/2 mutations (reviewed in 3). However, only a handful of these factors were confirmed and independently validated as “true modifiers” (4). The aim of the present study was to assess the putative modifier effect of 3,248 sequence alterations in 445 candidate genes on breast/ovarian cancer risk in 23,463 BRCA1 and BRCA2 mutation carriers.

Materials and methods

Recruitment and data collection

All study participants were women, >18 years old, carrying a deleterious germline mutation in either BRCA1 or BRCA2. DNA samples and phenotypic data were submitted by 54 study centers participating in the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) (5). Recruitment strategies, clinical, demographic, and phenotypic data collected from each participant, and quality control procedures, have previously been reported (4,5). All study participants took part in research studies at the parent institutions under ethically-approved protocols as detailed (4,5).

Sequence variants genotyped

DNA samples were genotyped using the custom Illumina iCOGS array which included 211,155 single nucleotide polymorphisms (SNPs) as previously described (http://www.nature.com/icogs/primer/cogs-project-and-design-of-the-icogs-array/; 6). We report results from 3,248 SNPs from 445 candidate genes proposed by 17 PIs (=projects). The rationale for selecting the SNPs or genes as candidate cancer risk modifiers in BRCA1 and BRCA2 mutation carriers is shown in Table 1. The list of SNPs included in the study and their gene location (if any) is provided in Supplementary Table 1. Genotyping quality control procedures were carried out as reported elsewhere (6).

Statistical analysis

Associations were evaluated within a retrospective cohort framework, by modeling the retrospective likelihood of the observed genotypes conditional on the disease phenotypes (4,7). The associations between genotype and breast or ovarian cancer risk were assessed using the 1 d.f. score test statistic based on this retrospective likelihood while accounting for the non-independence among related individuals (8). All analyses were stratified by country of residence and used calendar-year and cohort-specific breast and ovarian cancer incidence rates for BRCA1 and BRCA2 mutation carriers. Details are provided elsewhere (2).

Results

A total of 23,463 mutation carriers were included (15,252 BRCA1, 8,211 BRCA2 carriers), 12,127 with breast cancer (7,797 BRCA1, 4,330 BRCA2 carriers), 3,093 with ovarian cancer (2,462 BRCA1, 631 BRCA2 carriers), and 9,220 cancer-free carriers (5,788 BRCA1, 3,432 BRCA2 carriers). All 3,248 SNPs were tested as genetic risk modifiers for both breast...
and ovarian cancer in BRCA1 and BRCA2 mutation carriers depending on the selection rationale (Table 1). For each SNP, the number of individuals with genotype data, minor allele frequencies (MAF), values of the X² score test statistic, approximate hazard ratio (HR) estimates based on the score test statistic (7), overall P values and retrospective likelihood HR are shown in Supplementary Table 2. Since project 12 was based on the hypothesis that estrogens contribute to breast cancer pathogenesis, these 139 SNPs were stratified by somatic estrogen receptor status (Supplementary Table 3). None of the SNPs tested showed significant evidence of association with breast and/or ovarian cancer risk, as a single tested variant or after adjusting for mutiple testing. Indeed, there were fewer associations at a nominal P<0.05 or P<0.01 than would be expected by chance (Table 2).

Discussion

In this study, there were no discernible effects for the genotyped SNPs on either breast or ovarian cancer risk in BRCA1 or BRCA2 mutation carriers. Despite the lack of evidence of association between these specific variants and breast/ovarian cancer risk for BRCA1/BRCA2 mutation carriers, these genes may still modify cancer risk by other sequence alterations that are not represented on the iCOGS platform, by epigenetic alterations in gene expression, or in combination and interaction with other polymorphisms, that in concert have an overall effect on cancer risk.

In conclusion, the genotyped SNPs in the candidate modifier genes evaluated here have no major role in breast or ovarian cancer risk modification in either BRCA1 or BRCA2 mutation carriers. Our results suggest that a candidate gene approach where the selected SNPs have little a priori biological plausibility is of limited value in identifying modifier genes, unlike agnostic genome-wide associations which have been more successful (8). Applying more advanced technologies, (whole exome/genome sequencing) and targeting phenotypically distinct mutation carriers may also offer further insights into modifier genes’ identity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References


Description of the 17 projects included in the study.

Table 1

<table>
<thead>
<tr>
<th>Project</th>
<th>Rationale for testing SNPs as risk modifiers for breast cancer and ovarian cancer in BRCA-mutation carriers</th>
<th>Number of SNPs included</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>X chromosome SNPs shown to be associated with risk of breast cancer in the CGEMS breast cancer study were considered.</td>
<td>11</td>
<td>Hunter DJ et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet. 2007 Jul;39(7):870–4.</td>
</tr>
<tr>
<td>3</td>
<td>Previous data suggested that the “del” allele of rs3834129 was associated with increased breast cancer risk in BRCA1-mutation carriers.</td>
<td>1</td>
<td>Catucci I et al. The CASP8 rs3834129 polymorphism and breast cancer risk in BRCA1 mutation carriers. Breast Cancer Res Treat. 2011 Feb;125(3):855-60.</td>
</tr>
<tr>
<td>4</td>
<td>Search for risk modifiers of BRCA1 5382insC-mutation carriers was performed by a pooled GWAS in 124 women diagnosed with breast cancer (&lt;45 years) and 119 unaffected controls (&gt;50 years at last follow up) from Poland. The highest-ranked SNPs from the pooled GWAS were selected.</td>
<td>137</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>The proposed SNPs are related to genes in regulatory T-cell (Treg) cell and myeloid derived suppressor cell (MDSC) pathways. Both pathways play a role in cancer immunosuppression.</td>
<td>2637</td>
<td>Schreiber RD et al. Cancer immunoediting; integrating immunity’s roles in cancer suppression and promotion. Science. 2011;331(6024):1565–1570</td>
</tr>
<tr>
<td>Project</td>
<td>Rationale for testing SNPs as risk modifiers for breast cancer and ovarian cancer in BRCA-mutation carriers</td>
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</tr>
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<tr>
<td>12</td>
<td>Steroid hormones such as estrogens play an important role in the etiology of breast cancer contributing to tumor growth by promoting cell proliferation. SNPs in candidate genes involved in sex steroid metabolism were considered. The SNPs were tested also as breast cancer risk modifiers considering estrogen receptor status of BRCA-mutation carriers (see Supplementary Table 3)</td>
<td>139</td>
<td>Labrie F et al. Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. Endocr Rev. 2003;24(2):152–82.</td>
</tr>
<tr>
<td>13</td>
<td>RAD51C is a breast cancer gene. SNPs located within, or in close proximity to RAD51C were selected.</td>
<td>17</td>
<td>Meindl A et al. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nat Genet. 2010;42(5):410–4.</td>
</tr>
<tr>
<td>14</td>
<td>The highest-ranked SNPs from a GWAS based on 700 hereditary breast cancer cases and 1,200 controls were selected.</td>
<td>142</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>The rs10895068 SNP in the promoter of the progesterone receptor (PR) gene (+331G/A) has been reported to be associated with endometrial cancer risk. Our previous study in 220 patients from BC and OC families showed a marginal association of the +331A allele with OC risk. This SNP was tested only as modifier of ovarian cancer risk.</td>
<td>1</td>
<td>Vivo ID et al. A functional polymorphism in the promoter of the progesterone receptor gene associated with endometrial cancer risk. Proc Natl Acad Sci U S A. 2002;99(19):12263–12268. Romano A et al. Impact of two functional progesterone receptor polymorphisms (PRP): +331G/A and PROGINS on the cancer risks in familial breast/ovarian cancer. Open Cancer J. 2007;1:1–8.</td>
</tr>
<tr>
<td>17</td>
<td>The proposed SNPs were selected according to the hypothesis that different levels of expression of the remaining normal allele in BRCA2 mutation carriers may be associated with variable penetrance of BRCA2 mutations.</td>
<td>24</td>
<td>Maia AT et al. Effects of BRCA2 cis-regulation in normal breast and cancer risk amongst BRCA2 mutation carriers. Breast Cancer Res. 2012;14(2):R63</td>
</tr>
</tbody>
</table>
Table 2

Observed and expected number of SNPs with p-values <0.05 and <0.01

<table>
<thead>
<tr>
<th>Category</th>
<th>Tumor</th>
<th>Number of SNPs tested</th>
<th>Number of SNPs with p-value&lt;0.01 (expected)</th>
<th>Number of SNPs with p-value&lt;0.05 (expected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>BrCa</td>
<td>3232</td>
<td>25 (32)</td>
<td>202 (162)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>OvCa</td>
<td>3160</td>
<td>13 (32)</td>
<td>146 (158)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>BrCa</td>
<td>3230</td>
<td>5 (32)</td>
<td>96 (161)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>OvCa</td>
<td>3157</td>
<td>6 (32)</td>
<td>131 (159)</td>
</tr>
</tbody>
</table>

* Not all the 3,248 SNPs were tested in each category/tumor group