BRCA1 MUTATION LOCATION AND RISK OF SUBSEQUENT BREAST OR OVARIAN CANCER AFTER BREAST CANCER.

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In draft.
ABSTRACT

Background: Women carrying a germline mutation in the BRCA1 gene face a high lifetime risk of developing breast cancer or ovarian cancer. Our aim was to investigate whether specific BRCA1 mutation regions are associated with different contralateral breast or ovarian cancer risks after a primary breast cancer.

Methods: Analyses included >6,000 patients with invasive early breast cancer diagnosed age <50 years between 1970-2003 with comprehensive longitudinal data, including >200 BRCA1 mutation carriers. Absolute risks for a second contralateral breast cancer and ovarian cancer after breast cancer related to BRCA1 regions (in exon 11) were calculated using cumulative incidence curves.

Results: BRCA1 mutations located in the Ovarian Cancer Cluster Region (OCCR) gave a relative 2-4 times higher ovarian cancer risk compared to the other regions; 10-year cumulative absolute risk: 13.5%(95%CI=5.4-25.2). No associations between specific BRCA1 regions and contralateral breast cancer were observed.

Conclusion: The OCCR mutation spectrum identifies a subgroup of BRCA1-associated breast cancer patients with a relatively higher risk of subsequent ovarian cancer.
INTRODUCTION

Hereditary BRCA1/2 mutations lead to highly increased breast and ovarian cancer risks (Brohet et al., 2014; Mavaddat et al., 2013). Very limited information exists regarding the relationship between specific BRCA1/2 germline mutations and cancer risks (Gayther et al., 1997; Rebbeck et al., 2015; Teixeira et al., 2015; Thompson et al., 2002). Recently, mutation-specific primary breast compared to ovarian cancer risks for BRCA1/2-mutation carriers were described (Rebbeck et al., 2015). In the BRCA1 gene, two Breast Cancer Cluster Regions (BCCRs) and one Ovarian Cancer Cluster Region (OCCR) were identified, with different associated relative risks of breast cancer versus ovarian cancer (BCCR1: 1.46, 95% CI=1.22-1.74; BCCR2: 1.34, 95% CI=1.01-1.78; OCCR: 0.62, 95% CI=0.56-0.70). Breast cancer patients identified as BRCA1-mutation carriers face decisions regarding their breast cancer treatment, follow-up, and/or (timing of) risk-reducing surgeries. Our aim was to investigate whether BCCRs and OCCR are associated with different contralateral breast or ovarian cancer risks after a primary breast cancer.

MATERIALS AND METHODS

Details of the study design and data collection have been published previously (van den Broek et al., 2016). In short, the study comprised an unselected, i.e. not family or clinical genetic center-based, consecutive series of 7,403 female patients with invasive breast cancer diagnosed before age 50 years without a previous cancer diagnosis. For 88% (N=6,484) of the total cohort we were able to collect germline DNA of sufficient quality, mainly using formalin-fixed paraffin-embedded normal tissue from hospital archives. This assured including patients irrespective of vital status, avoiding selection and survivor bias. We tested a panel of most prevalent Dutch BRCA1/2 mutations, i.e., 92 variants representing approximately 61% of the BRCA1/2 mutations prevalent in families in the Netherlands. We used allelic discrimination for substitutions and fragment length analyses for deletions and insertions; Sanger sequencing was used for confirmation of mutations (van den Broek et al., 2016).

Within the 210 (3.2%) identified BRCA1 mutation carriers, we defined three groups following the mutation region definitions by (Rebbeck et al., 2015): BRCA1 mutations located within no specific region; BRCA1 mutations located within the OCCR; and BRCA1 mutations located within the BCCR2 (no mutations located in the BCCR1 were tested).

Statistical analyses were performed similarly to those described previously (van den Broek et al., 2016); except that BRCA2 mutation carriers were excluded since numbers were
small (N=75) and no ovarian cancers were observed. In short, absolute risk estimates were
derived using cumulative incidence curves accounting for competing risks, and compared
using Gray’s test (Gray, 1988). Patients without follow-up (N=6) and patients with metasta-
sis at diagnosis (defined as detected within 3 months after primary diagnosis, N=125) were
excluded. For the contralateral breast cancer analyses time at risk started 3 months after
the diagnosis of the first breast cancer and ended at the date of diagnosis of contralateral
breast cancer, contralateral mastectomy, first distant metastases, death, or date of most
recent follow-up information, whichever came first. For the ovarian cancer analyses time
at risk started directly after the diagnosis of the first breast cancer and ended at the date
of diagnosis of ovarian cancer, (salpingho-)oophorectomy, first distant metastases, death,
or date of most recent follow-up information, whichever came first. In both analyses first
distant metastasis and death were taken into account as competing events.

The two analyses were performed with appropriate exclusions based on the specific
end-point. In the analyses for contralateral breast cancer 6,223 patients were included;
patients with synchronous bilateral breast cancer (contralateral breast cancer within 3
months; N=45), and patients who died or were lost to follow-up within 3 months (N=10)
were excluded. In the analyses for ovarian cancer 6,277 patients were included; patients
with ovarian cancer at breast cancer diagnosis (N=1).

The secondary use of long-term stored tissue samples and clinical data in this
study was in accordance with the Dutch codes of conduct (http://www.federa.org/
codes-conduct) (Riegman & van Veen, 2011) and approved by the review boards of the
participating institutions.

RESULTS

Numbers of events and specific details of the patients included in the contralateral
breast cancer analyses are shown in Figure 1A. The 10-year cumulative risks of con-
tralateral breast cancer for the three BRCA1 regions did not differ (Figure 1B): 19.3%
(95% CI=9.5-31.6) for carriers with BRCA1 mutations located within no specific region;
21.7% (95% CI=13.3-31.3) for carriers with mutations in the BCCR2; and 21.9% (95%
CI=11.8-34.1) for carriers with mutations in the OCCR. In line with our previous report
about an increased risk of contralateral breast cancer in BRCA1/2 mutation carriers
(van den Broek et al, 2016), contralateral breast cancer risks were significantly higher
in all three specific mutation groups of BRCA1-mutation carriers compared to non-
carriers.
BRCA1 mutation location and contralateral breast and ovarian cancer risk

Figure 1: Numbers of patients and specific events (A) and cumulative risks for contralateral breast cancer for BRCA1-mutation carriers, stratified by location of the mutations (BRCA1-OCCR vs BRCA1-ext: p=0.85; BRCA1-BCCR2 vs BRCA1-ext: p=0.92; BRCA1-BCCR2 vs BRCA1-OCCR: p=0.91), and non-carriers, up to 15 years (B). BRCA1-ext: carriers with BRCA1 mutations located within no specific region; BRCA1-OCCR: carriers with BRCA1 mutations located within the OCCR; BRCA1-BCCR2: carriers with BRCA1 mutations located within the BCCR2; a,bUnless otherwise specified; cLeft censored because of contralateral mastectomy before or within 3 months after the diagnoses of the first breast cancer.

Numbers of events and specific details of the patients included in the ovarian cancer analyses are shown in Figure 2A. The 10-year cumulative ovarian cancer risk for BRCA1-mutation carriers was 6.3% (95% CI=3.3-10.6) compared to 0.4% (95% CI=0.2-0.5) in non-carriers (p<0.001). The 10-year cumulative ovarian cancer risk stratified by BRCA1 mutation location was statistically significantly higher in carriers with mutations located...
within the OCCR: 13.5% (95% CI=5.4-25.2) compared to carriers with mutations located within the BCCR2: 2.4% (95% CI=0.5-7.7, p=0.003), and non-significantly higher compared to those with mutations within no specific region: 6.0% (95% CI=1.6-14.9, p=0.13) (Figure 2B).

<table>
<thead>
<tr>
<th></th>
<th>Non-carriers</th>
<th>BRCA1-ext</th>
<th>BRCA1-OCCR</th>
<th>BRCA1-BCCR2</th>
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<tr>
<td></td>
<td>N*</td>
<td>%*</td>
<td>N*</td>
<td>%*</td>
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<tr>
<td>Breast cancer patients</td>
<td>6070</td>
<td>56 (5.4)</td>
<td>56</td>
<td>6 (6.7)</td>
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<td>Median age, years (SD)</td>
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<td>412</td>
<td>356</td>
<td>965</td>
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<td>Ovarian cancer rate/10,000 person-years</td>
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<td>8.9</td>
<td>9</td>
<td>16.4</td>
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<td>7.3</td>
<td>53</td>
<td>5.3</td>
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<tr>
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<td>15.2</td>
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<tr>
<td>(Salpingo-)oophorectomy</td>
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<td>8.0</td>
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<td>44.6</td>
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<td>Alive at end of follow-up</td>
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<td>49.3</td>
<td>7</td>
<td>12.5</td>
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Figure 2. Numbers of patients and specific events (A) and cumulative risks for ovarian cancer for BRCA1-mutation carriers, stratified by location of the mutations (BRCA1-OCCR vs BRCA1-ext: p=0.13; BRCA1-BCCR2 vs BRCA1-ext: p=0.26; BRCA1-OCCR vs BRCA1-BCCR2: p=0.003), and non-carriers, up to 15 years (B).

BRCA1-ext: carriers with BRCA1 mutations located within no specific region; BRCA1-OCCR: carriers with BRCA1 mutations located within the OCCR; BRCA1-BCCR2: carriers with BRCA1 mutations located within the BCCR2; a,bUnless otherwise specified; cLeft censored because of (salpingo-) oophorectomy before or at the diagnosis of the first breast cancer.
DISCUSSION

In prospective analyses of a hospital-based cohort of young breast cancer patients, we found that pathogenic *BRCA1* germline mutations located in the OCCR region of the *BRCA1* gene lead to an increased risk of ovarian cancer. This specific OCCR region was identified in *BRCA1*-mutation carriers from the clinical genetic setting using a analysis based on ratios of breast versus ovarian cancers (Rebbeck et al., 2015), and was consistent with prior reports of the OCCR region being located in exon 11 of the *BRCA1* gene (Ramus & Gayther, 2009; Teixeira et al., 2015; Thompson et al., 2002). Our analyses, performed in a consecutive series of breast cancer patients unselected for family history with comprehensive and longitudinal data available, now show that these specific regions are also associated with differential risk of ovarian cancer after breast cancer. The number ovarian cancer events in our cohort is small, and larger cohort studies are needed to repeat our results, but we performed stringent analyses adjusting for risk-reducing surgeries.

(Rebbeck et al., 2015) also showed an association between the BCCR2 and a relatively higher risk of breast cancer. We did not observe such an increased risk for contralateral breast cancer. This is not necessarily conflicting since our patients had already developed a first breast cancer and treatment therefore may have affected the risk for a contralateral breast cancer, and we had limited power.

In conclusion, the OCCR mutation spectrum identifies a subgroup of *BRCA1*-associated breast cancer patients with a relatively higher risk of ovarian cancer during follow-up. These results contribute to further knowledge regarding differential ovarian cancer risks for high-risk women, and, with further validation, may help to improve tailored counseling regarding the optimal decision concerning (the timing of) risk-reducing salpingohooophorectomy for ovarian cancer.

ACKNOWLEDGEMENTS

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REFERENCES


