Chapter 2

Proven non-carriers in BRCA1/2 families have an earlier age of onset of breast cancer

Janet R. Vos
Geertruida H. de Bock
Natalia Teixeira
Dorina M. van der Kolk
Liesbeth Jansen
Marian J.E. Mourits
Jan C. Oosterwijk

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Abstract

Background
Risk estimates for proven non-carriers in BRCA mutation families are inconsistent for breast cancer and lacking for ovarian cancer. We aimed to assess the age-related risks for breast and ovarian cancer for proven non-carriers in these families.

Methods
A consecutive cohort study ascertained 464 proven non-carriers who had a first-degree relative with a pathogenic BRCA mutation. Kaplan–Meier analyses were used to estimate the age-related cancer risks, and we calculated standardised incidence ratios.

Results
In the 464 non-carriers, 17 breast cancers and two ovarian cancers were detected at a mean age of 47 years (95% confidence interval (CI) 32–61) and 49 years (95% CI 32–67), respectively. Overall, by the age of 50, the breast and ovarian cancer risks among non-carriers were 6.4% (95% CI 2.9–9.8%) and 0.4% (95% CI 0–1.3%), of which the breast cancer risk was statistically significantly higher than the risk in the general population. In particular, the number of breast cancers among non-carriers in BRCA1 families was higher than expected for the general population (standardised incidence ratio (SIR) 2.0, 95% CI 1.1–3.3). In the BRCA1 cohort, the mean number of breast cancer cases was higher in families in which non-carriers were diagnosed before the age of 50 ($p = 0.04$).

Conclusion
The age at diagnosis of breast cancer in non-carriers in BRCA mutation families is younger than expected, yielding an increased risk in the fifth decade. This effect is most evident in BRCA1 families. If our results are confirmed by others, this could affect the advice given on breast cancer screening to proven non-carriers between the age of 40 and 50 in such families.
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**Introduction**

Female carriers of a pathogenic mutation in the *BRCA1/2* genes have a high risk of developing breast and ovarian cancer: lifetime risks range from 45% to 88% and 11% to 59%, respectively.\(^1\)\(^-\)\(^6\) Carriers are therefore enrolled in an intensive screening program and may opt to have preventive surgery. In the Netherlands, this program runs from the age of 25–60 years and consists of an annual physical examination, annual breast MRI and, from the age of 30, annual mammography.\(^7\) There is no screening program for ovarian cancer, but *BRCA1/2* mutation carriers from the age of 35–40 may opt for risk-reducing salpingo-oophorectomy (RRSO).

It has been assumed that women who tested negative for their family-specific *BRCA* mutation were not at an increased risk.\(^8\)\(^-\)\(^11\) They are dismissed from intensive screening and referred to our national breast cancer screening program, which consists of biennial mammography from the age of 50–75 years. However, the cancer risk of these proven non-carriers in *BRCA*-positive families is under debate\(^12\) and several studies have published contradictory results on their residual breast cancer risk.\(^8\)\(^-\)\(^11\), \(^13\)\(^-\)\(^16\) The reported risk ratios ranges from 0.39 to 5.3, while there are no figures available for residual ovarian cancer risk.

It is thus uncertain whether non-carriers in *BRCA*-positive families are rightly being advised to stop screening and to wait until they can enrol in the national program, and we do not know what advice to give them regarding their possible ovarian cancer risk. Our aim was to evaluate the breast and ovarian cancer risks for proven non-carriers who have a first-degree relative with a pathogenic *BRCA* mutation.

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**Methods**

Our family cancer clinic in the University Medical Center Groningen provides genetic counselling and screening to women who may carry a *BRCA* mutation based on their personal and/or family history. If the patient or family fulfils the Dutch criteria for genetic testing, comprehensive *BRCA1* and *BRCA2* mutation testing is performed in one or more index cases.\(^17\) Once a pathogenic *BRCA* mutation has been detected, targeted genetic testing for this mutation is offered to all relatives, using a cascade protocol.\(^18\)

Information was collected up to March 2008 and previously used to calculate the breast and ovarian cancer penetrance for *BRCA* mutation families;\(^3\) this information was updated up to September 2011 for the current study. Data were retrieved from patients’ medical records and entered into a separate, anonymous, password-protected database. Under the Dutch law, this means no further approval from our institution’s Ethics Review Board was needed.

For all women, we collected information on the date of birth and death or last contact, as well as data on the familial gene mutation, their breast- and ovarian cancer status, and if and when a risk-reducing mastectomy (RRM) and/or RRSO had been performed. Data about breast- and ovarian cancer in the general population was
obtained from the Dutch Comprehensive Cancer Centre.\textsuperscript{19, 20}

All analyses were performed using PASW Statistics 18.0 software, and statistical significance was defined as $p < 0.05$. Descriptive statistics were applied to analyse patient characteristics, differences in continuous and categorical variables were tested two-sided with the Mann–Whitney U-test and Fishers’ exact test, respectively. Kaplan–Meier survival analyses were used to calculate the cumulative incidence rates. To calculate the breast cancer risk, right-censoring was applied at the age of RRM (N = 1), the age at RRSO if performed before the age of 50 (N = 4), at the last contact, or age at death. In the ovarian cancer risk analyses, women were censored at the age at RRSO (N = 4), at the last contact, or age at death. Standardised incidence ratios (SIRs) were calculated for the age-specific breast cancer incidence, both with and without stratification by the \textit{BRCA} gene. The numbers of observed cases were compared with the numbers of expected cases, which were calculated using data from the Dutch Comprehensive Cancer Centre.\textsuperscript{19, 20} To account for possible ascertainment bias, SIRs were also calculated for the group of proven non-carriers expanded with the group of non-tested women. In unaffected women, the probability of testing negative increases with advancing age, so we divided women into 10-year age groups that had different probabilities of being negative. The numbers in each age group were multiplied by the probability of being negative and then added together to obtain the estimated number that would have tested negative.\textsuperscript{14}

\textbf{Results}

In 365 \textit{BRCA}-positive families (219 \textit{BRCA1} and 146 \textit{BRCA2} families), 1524 women were tested for a \textit{BRCA1} or \textit{BRCA2} mutation. Of these, 464 women tested negative for their family-specific \textit{BRCA} mutation and were included in this study. In total there were 700 untested first-degree female relatives of 20 years or older, of whom 184 (26\%) developed breast cancer.

With a mean age at last contact of 44.9 years for the group of non-carriers, 17 women had been diagnosed with breast cancer. Thirteen of these cases were among 283 non-carriers in \textit{BRCA1} families (one case was bilateral) and four were among 181 non-carriers in \textit{BRCA2} families. For the 17 cases the mean age at diagnosis was 46.5 years (standard deviation (SD) 7.0): 44.8 years (SD 7.2) in \textit{BRCA1} families and 51.9 years (SD 2.9) in \textit{BRCA2} families ($p = 0.045$). This mean age at diagnosis was lower than the mean age seen in the general population of 61.9 years (SD 14.3), but the difference was not significant ($p = 0.17$).\textsuperscript{3}

Two cases of ovarian cancer had been detected, both in non-carriers in \textit{BRCA1} families. Their ages at diagnosis were 43 and 55 years, which were much lower than the mean age in the general population (65.5, SD 13.7).

The overall breast cancer risk for non-carriers in \textit{BRCA2} families was lower than in \textit{BRCA1} families, but this was not significant (hazard ratio = 0.44 (95\% confidence interval (CI) 0.14–1.35), $p = 0.15$). By the age of 60, the non-carriers had a breast cancer risk of 9.5\% (95\% CI 5.0–14.1\%), whereas women in the general population had a risk
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Table 1. Cumulative incidence of breast and ovarian cancer among proven BRCA non-carriers and the general population.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Breast cancer</th>
<th>Ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proven non-carriers</td>
<td>General population</td>
</tr>
<tr>
<td>40</td>
<td>1.3 (0.0–2.5)</td>
<td>0.6</td>
</tr>
<tr>
<td>50</td>
<td>6.4 (2.9–9.8)</td>
<td>2.5</td>
</tr>
<tr>
<td>60</td>
<td>9.5 (5.0–14)</td>
<td>5.2</td>
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</table>

* 95% confidence interval shown in brackets

Figure 1. Cumulative incidence of breast cancer (A) and ovarian cancer (B) for non-carriers (with 95% confidence interval) and age-matched women from the general population.

of 5.2% (Table 1).19 At all ages, the breast cancer risk was higher in the proven non-carriers than in the age-matched cohort from the general population (Fig. 1). By the age of 50 the breast cancer risk was significantly higher; the expected cumulative incidence was outside the confidence interval of the observed cumulative incidence. The ovarian cancer risk was slightly higher in non-carriers than in the general population from age 50 upwards, but this was not significant (Table 1).

We compared the observed number of breast cancers to the expected number of cases in the general population (Table 2). There were significantly more cases observed in the non-carriers in BRCA1 families in the age groups under 50 years: 30–39 years SIR 11 (95% CI 3.0–29) and 40–49 years SIR 4.5 (95% CI 1.8–9.2). In non-carriers in BRCA2 families, the number of observed cases was not significantly higher than expected. Except for the significantly raised SIRs in the BRCA2 group 40–49 years and the total BRCA group, the SIRs did not change significantly when the calculation was based on non-carriers and a proportion of the 700 women not tested (Table 2).
The 19 symptomatic non-carriers came from 14 BRCA1 and 4 BRCA2 families. In 204 BRCA families, all the proven non-carriers were still so far free of breast and/or ovarian cancer, while another 143 BRCA families contained no known non-carriers. To assess the difference in cancer incidence due to genetic modifiers and/or environmental factors, we compared the relatives in BRCA families with symptomatic non-carriers to those families without symptomatic non-carriers (Table 3). We observed no differences in the numbers of cases or mean age at diagnosis, or in the cancer risk for either all family members or for the carriers alone. However, among the BRCA1 families, the number of breast cancers was significantly higher in families with non-carriers diagnosed with breast cancer before the age of 50 than in families with non-carriers affected after the age of 50, or in families with no affected non-carriers.

**Discussion**

We investigated the occurrence of breast and ovarian cancer in 464 proven non-carriers in BRCA families in a consecutive cohort study with a clinic-based ascertainment. We show that the cumulative incidence of breast cancer in proven non-carriers in their fifth decade is 2.6 times higher than in the general population, with a SIR of 3.5 (95% CI 1.6–6.7) and a mean age at diagnosis that was 15 years earlier. This study is the first to assess ovarian cancer risks for proven non-carriers. Only two cases were diagnosed at ages much younger than the mean age at diagnosis in the general population.

Previously published estimates on breast cancer risk in non-carriers in the BRCA families vary widely. We found an increased cancer risk for proven non-carriers at all ages compared with women from the general population. This is in line with three other clinic-based studies from England, Canada and Poland that reported a significantly increased risk of at least twofold. However, four other studies (two clinic-based studies from the United States and one from Australia, and one population-based study from the United States) report contradictory results that show that the risk of proven non-carriers is not increased or at least not twice as high as in the general population. Possible explanations may be differences in: (1) ascertainment, (2) study design, or (3) national screening protocols. First, studies with ascertainment by a family cancer clinic will probably result in higher risk estimates, since families referred to these clinics have a stronger history of breast cancer than the general population. Second, although a prospective study design is favored, it might result in lower risk estimates as a substantial number of the family members have already been diagnosed before visiting the family cancer clinic, and non-symptomatic proven non-carriers are no longer followed-up in such clinics. As an illustration, when we applied left-censoring at the moment of the individual’s DNA test, the follow-up time was short and only a few breast cancer cases were observed in this period. Third, countries with more stringent inclusion criteria for genetic counselling or those in which additional screening for non-carriers is readily available will probably show a larger risk difference between non-carriers in proven BRCA families and the general population.
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Table 2. Standardised incidence ratios of breast cancer by BRCA gene and age group in (A) non-carriers and (B) non-carriers and assumed non-carriers.

<table>
<thead>
<tr>
<th>Age</th>
<th>BRCA1 Obs.</th>
<th>BRCA1 Exp.</th>
<th>BRCA1 SIR</th>
<th>BRCA1 95% CI</th>
<th>BRCA2 Obs.</th>
<th>BRCA2 Exp.</th>
<th>BRCA2 SIR</th>
<th>BRCA2 95% CI</th>
<th>BRCA total Obs.</th>
<th>BRCA total Exp.</th>
<th>BRCA total SIR</th>
<th>BRCA total 95% CI</th>
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<td></td>
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<tr>
<td>(A) Non-carriers</td>
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</tr>
<tr>
<td>30–39</td>
<td>4</td>
<td>0.4</td>
<td>11</td>
<td>3.0–29</td>
<td>0</td>
<td>0.3</td>
<td>–</td>
<td>–</td>
<td>4</td>
<td>0.6</td>
<td>6.4</td>
<td>1.7–16</td>
</tr>
<tr>
<td>40–49</td>
<td>7</td>
<td>1.6</td>
<td>4.5</td>
<td>1.8–9.2</td>
<td>2</td>
<td>0.9</td>
<td>2.1</td>
<td>0.3–7.6</td>
<td>9</td>
<td>2.6</td>
<td>3.5</td>
<td>1.6–6.7</td>
</tr>
<tr>
<td>50–59</td>
<td>2</td>
<td>2.6</td>
<td>0.8</td>
<td>0.1–2.8</td>
<td>2</td>
<td>1.8</td>
<td>1.2</td>
<td>0.1–4.4</td>
<td>4</td>
<td>4.5</td>
<td>0.9</td>
<td>0.2–2.3</td>
</tr>
<tr>
<td>Total b</td>
<td>13</td>
<td>6.6</td>
<td>2.0</td>
<td>1.1–3.3</td>
<td>4</td>
<td>4.7</td>
<td>0.8</td>
<td>0.2–2.1</td>
<td>17</td>
<td>11.6</td>
<td>1.5</td>
<td>0.9–2.3</td>
</tr>
<tr>
<td>(B) Non-carriers and not-tested women</td>
<td></td>
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<tr>
<td>30–39</td>
<td>6.5</td>
<td>0.6</td>
<td>11</td>
<td>5.8–19</td>
<td>0.5</td>
<td>0.3</td>
<td>1.6</td>
<td>0.1–11</td>
<td>7</td>
<td>0.9</td>
<td>7.5</td>
<td>3.7–12</td>
</tr>
<tr>
<td>40–49</td>
<td>8.6</td>
<td>1.5</td>
<td>3.4</td>
<td>1.9–5.7</td>
<td>4.5</td>
<td>1.6</td>
<td>2.8</td>
<td>1.1–4.7</td>
<td>13</td>
<td>4.2</td>
<td>3.1</td>
<td>2.0–5.0</td>
</tr>
<tr>
<td>50–59</td>
<td>4</td>
<td>4.3</td>
<td>0.9</td>
<td>0.4–1.7</td>
<td>4</td>
<td>3.0</td>
<td>1.3</td>
<td>0.9–3.2</td>
<td>8</td>
<td>7.6</td>
<td>1.1</td>
<td>0.6–1.7</td>
</tr>
<tr>
<td>Total b</td>
<td>19</td>
<td>11</td>
<td>1.7</td>
<td>1.0–2.5</td>
<td>9.1</td>
<td>8.3</td>
<td>1.1</td>
<td>0.7–1.9</td>
<td>28</td>
<td>20</td>
<td>1.4</td>
<td>1.0–2.0</td>
</tr>
</tbody>
</table>

Abbreviations: obs. observed; exp. expected; SIR standardised incidence ratio; CI confidence interval

a No breast cancers were observed after the age of 59 years
b Ages: 20–69 years

Table 3. Family history of cancer in BRCA carriers and relatives at 50% risk but not tested, in families with and without affected proven non-carriers.

<table>
<thead>
<tr>
<th>BRCA families a</th>
<th>BRCA1 families b</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relatives of affected non-carriers N = 115</td>
<td>Relatives of only non-affected non-carriers N = 1174</td>
<td>p</td>
<td>Relatives of affected non-carriers with BC &lt; 50 years N = 75</td>
</tr>
</tbody>
</table>

Mean age (standard deviation, SD)

<table>
<thead>
<tr>
<th>Mean age</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>46 (12)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>51 (11)</td>
</tr>
</tbody>
</table>

Number (%)

<table>
<thead>
<tr>
<th>Number (%)</th>
<th>p</th>
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<tbody>
<tr>
<td>Breast cancers</td>
<td>48 (42%)</td>
</tr>
<tr>
<td>Ovarian cancers</td>
<td>21 (18%)</td>
</tr>
</tbody>
</table>

Abbreviations: BC, breast cancer.
a Comparison of BRCA families with affected proven non-carriers with a diagnosis of breast or ovarian cancer and families with only proven non-carriers with no diagnosis of breast or ovarian cancer.
b Comparison of BRCA1 families with non-carriers with a breast cancer diagnosis before the age of 50, on the one hand, and BRCA1 families with affected non-carriers at over 50 and BRCA1 families with no affected non-carriers, on the other hand.
The younger age of proven non-carriers at diagnosis of breast cancer has not been discussed before. Their mean age of breast cancer diagnosis (46.5 years, SD 7.0) is considerably lower than the mean age for the general Dutch population (61.9 years, SD 14.3). This is mainly due to the high number of diagnoses in non-carriers in \textit{BRCA1} families in the 30–39 and 40–49 year age groups, resulting in SIRs of 11 and 4.5, respectively. Other studies in non-carriers have reported mean ages at diagnosis between 44.3 and 50.9 years. The different study approaches may explain the differences found in the mean age at breast cancer diagnosis. The figures we present here are based on a cohort study in a family cancer clinic setting, which included all non-carriers with a first-degree relative carrying a \textit{BRCA} mutation. To date, the lowest mean age at diagnosis, 44.3 years, was reported in a clinic-based retrospective cohort study that consisted of only Ashkenazi Jewish families, which might explain the lower mean age at diagnosis. The highest mean age at diagnosis, 50.9 years, was reported in a population-based study in first-degree relatives in \textit{BRCA}-negative families, but it also reported mean ages of 42.1 and 44.5 years in \textit{BRCA1} - and \textit{BRCA2} -positive families, respectively.

Recent large studies have shown that the genetic variability in breast and ovarian cancer risks can be partly explained by common modifier alleles. Therefore, it is likely that a residual increased risk of breast cancer in proven non-carriers from \textit{BRCA} families will be due to the presence of other familial, predisposing low-risk alleles and/or \textit{BRCA} penetrance modifiers. We assumed that in families with such penetrance-increasing modifiers, the percentage of symptomatic mutation carriers and the percentage of bilateral cases would be higher, and that their mean age at diagnosis would be lower. We therefore compared these parameters between the families with symptomatic non-carriers and the families with only non-symptomatic non-carriers and found significantly more cases of breast cancer in \textit{BRCA1} families with non-carriers diagnosed before the age of 50 than in all other \textit{BRCA1} families with proven non-carriers. Future analyses of genetic modifiers and/or environmental factors in our cohort may explain these findings, but at the moment we have no overall explanation for the increased risk seen in young non-carriers from \textit{BRCA1} families.

Our study has various strengths, for example, its design as a consecutive cohort with a uniform, clinic-based ascertainment. We consider data obtained from non-carrier populations that are ascertained at family cancer clinics to be different to population-based data and, as such, more applicable to the genetic counselling setting. The women seen at the genetics clinics are the ones to whom the screening policies are actually applied and which are adapted according to their mutation status. Our study is the first to present an ovarian cancer risk assessment for proven non-carriers and the low risk figures appear to be reassuring.

Our study may have been affected by several limitations. First, it might be liable to recall bias because we used information on the family history of cancer reported by women during the course of regular care at the family cancer clinic. However, cases were validated by hospital records or information was provided by first-degree relatives, who are known to report their family cancer history accurately. If any bias is present, we are more likely to have under- than overestimated because relatives are more likely to under-report than to over-report cancer. Second, our study could be
subject to ascertainment bias. Once a non-symptomatic woman has tested negative, she is no longer monitored in a high-risk screening program, and BRCA2 families are less likely to be referred to a family clinic because of the older mean age at diagnosis of breast cancer. Both issues may lead to an under-reporting of cancer and consequently underestimate the cancer incidence in the study population and influence the mean age at diagnosis. However, there was only a minor significant change in the SIRs in the total BRCA group when first-degree relatives who had not been tested were included. Third, selection bias could play a role since no proven non-carriers were known in 143 BRCA families. However, we observed no other differences between these and the other BRCA families, so we would expect any potential effect to be small.

The results from our previous study\(^3\) and the current analyses show that female non-carriers in BRCA families are indeed at an increased risk of developing breast cancer from age 40 onwards, which is in line with the results of Smith et al.\(^{14}\) The number of observed cases in non-carriers in the age 40–49 group is significantly increased: SIR 3.5 (95% CI 1.6–6.7). Their risk increase is substantial and may justify starting screening from the age of 40,\(^{29}\) rather than waiting for the national screening protocol which starts at the age of 50.\(^{30,31}\) The two ovarian cancer cases occurred at an earlier age than seen in the general population, but the very low incidence means that there is no need for medical intervention. Further research into lifestyle and genetic factors is needed to explain the increased breast cancer risk seen in proven BRCA non-carriers.

**Acknowledgement**

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Chapter 2

References


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