Variation in mutation spectrum partly explains regional differences in the breast cancer risk of female *BRCA* mutation carriers in the Netherlands

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Running title

Regional breast cancer risk differences in *BRCA* carriers

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Competing interests

The authors declare that they have no competing interests.

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Abbreviations:

BRCA Breast cancer gene

HR	Hazard ratio
OCCR	Ovarian cancer cluster region
OR	Odds ratio
RRM	Risk-reducing mastectomy
RRSO	Risk-reducing salpingo-oophorectomy

Abstract

Background: We aimed to quantify previously observed relatively high cancer risks in *BRCA2* mutation carriers (*BRCA2* carriers) over 60 in the Northern Netherlands, and to analyze whether these could be explained by mutation spectrum or population background risk.

Methods: This consecutive cohort study included all known pathogenic *BRCA1/2* carriers in the Northern Netherlands (N = 1,050). Carrier and general reference populations were: *BRCA1/2* carriers in the rest of the Netherlands (N = 2,013) and the general population in both regions. Regional differences were assessed with hazard ratios (HRs) and odds ratios (ORs). HRs were adjusted for birth year and mutation spectrum.

Results: All *BRCA1* carriers and *BRCA2* carriers under age 60 had a significantly lower breast cancer risk in the Northern Netherlands, HRs were 0.66 and 0.64, respectively. Above age 60, the breast cancer risk in *BRCA2* carriers in the Northern Netherlands was higher than in the rest of the Netherlands (HR = 3.99, 95% CI 1.11-14.35). Adjustment for mutational spectrum changed the HRs for *BRCA1*, *BRCA2* <60 and *BRCA2* ≥60 years by -3%, +32% and +11% to 0.75, 0.50 and 2.61, respectively. There was no difference in background breast cancer incidence between the two regions (OR = 1.03, 95% CI 0.97-1.09).

Conclusions: Differences in mutation spectrum only partly explain the regional differences in breast cancer risk in *BRCA2* carriers, and for an even smaller part in *BRCA1* carriers.

Impact: The increased risk in *BRCA2* carriers over 60 may warrant extension of intensive breast screening beyond age 60.

Introduction

Breast cancer is the most prevalent type of cancer in women. In the Netherlands, women in the general population have a lifetime risk of approximately 11% of developing breast cancer and 1% of ovarian cancer (1), while carriers of a pathogenic *BRCA1* or *BRCA2* mutation have a much higher risk of developing breast and/or ovarian cancer. Two international meta-analyses have estimated a mean breast cancer risk by age 70 of 57% and 65% for *BRCA1*, and 45% and 49% for *BRCA2* mutation carriers (*BRCA1/2* carriers) (2, 3). Because referred hereditary cases may have an even higher lifetime risk (4), counseling of *BRCA1/2* carriers in the Netherlands is still based on an estimated lifetime breast cancer risk of 60-80% (5).

In a previous study in the Northern Netherlands, relatively high risks were observed in *BRCA* carriers, especially in *BRCA2* carriers over age 60 (6). The cumulative incidence for breast cancer by age 70 with inclusion of the index cases was 71% (95% CI 67-76%) in *BRCA1* carriers and 88% (95% CI 82-93%) in *BRCA2* carriers. After excluding the index cases, these risks were 60% (95% CI 55-66%) and 78% (95% CI 69-88%), respectively. These figures, both with and without index cases, are at the high end of published risk estimates in several studies (2, 3, 6-10). Especially, the risk in *BRCA2* carriers observed in the Northern Netherlands is high, i.e. higher than the Dutch risks used in counseling (5).

Potential explanations for regional or national differences in breast cancer incidence rates are differences in genetic and non-genetic population factors, such as founder mutations, genetic modifiers, demographic composition, and lifestyle factors, as well as differences in ascertainment and methodology (10-16). The contribution of (founder) mutations in the *BRCA* genes in the ovarian cancer cluster region (OCCR) could, for instance, affect regional risk differences, since mutations within the OCCR are associated with a lower breast cancer risk (17, 18). Regional differences in the population breast cancer incidence, i.e. difference in the baseline risk, could also affect risk differences among *BRCA1/2* carriers. We aimed to determine whether there are regional differences in the breast cancer risk in *BRCA1/2* carriers in the Netherlands, and if so, whether they could be explained by mutation spectrum or population background risk.

Materials and Methods

The study cohort

The Family Cancer Clinic of the University Medical Center Groningen (UMCG) provides multidisciplinary counseling to residents of the Northern Netherlands (19). This is a stable population (20), and therefore feasible to assess the role of mutation spectrum in the existence of regional risk differences (21). The population of the study cohort included all (N = 1,050) women with a pathogenic *BRCA* mutation (either by testing or obligate carriers, i.e. a women with a child as

well as a parent or sibling carrying a mutation) known at the UMCG by September 2011. Ascertainment and the DNA testing procedure were described previously (6). Women were included if they were born after 1910, and from a minimum age of 18 years. The index cases were women with: 1) the first detected *BRCA1/2* mutation in their family, or 2) the youngest age at diagnosis of breast or ovarian cancer when the first *BRCA1/2* mutation in the family was detected in multiple females at the same time. No index case was defined in the family, when the first mutation was detected in a male or an unaffected woman.

Data collection in the study cohort

In the previous study, data were collected on the occurrence of breast and ovarian cancer in *BRCA1/2* carriers in the Northern Netherlands and their first-degree relatives up to March 2008 (6). For the current study, the dataset was updated by retrieving the patient files, and extended to September 2011.The update included data of: known carriers (N=233), new carriers (N=122) in known families and carriers (N=442) in newly ascertained families.

Information on women seen at the clinic was collected from their clinical genetic records, which also contained extensive and regularly updated pedigree information. Information on obligate carriers was obtained, with consent, from medical records. For all the women included, we recorded: date of birth, date of last contact/death, and data on the familial gene mutation (according to HGVS nomenclature) and individual carrier status, date of breast cancer diagnosis, date of ovarian cancer diagnosis, date of risk-reducing mastectomy (RRM) and, date of risk-reducing salpingo-oophorectomy (RRSO). These data were retrieved from patients' medical records and entered into a separate, anonymous, password-protected database. According to Dutch law, this means no further approval from the Institutional Review Board was needed.

Reference populations

The carrier reference population consisted of all *BRCA1/2* carriers from the rest of the Netherlands as registered in the HEBON study (N = 2,013), a national cohort of *BRCA1/2* families up to 2006 (10, 22). The general reference populations were the general population from the Northern Netherlands and the general population in the rest of the Netherlands. Data on the population background risk, i.e. age-related breast cancer incidence, in both regions were collected from the Dutch Comprehensive Cancer Centre (CCN/IKNL) for the period 2005-2010 (1).

Statistical analysis

In the analyses, obligate carriers and proven carriers were considered as one group. The mutation spectrum was assessed by stratifying the mutations for both genes in three previously defined groups: 5' to the OCCR, in the OCCR, and 3' to the OCCR (10, 17, 18). The *BRCA1* OCCR was defined as nucleotides 2282 to 4071, and the *BRCA2* OCCR as nucleotides 2831 to 6401 (10, 17, 18). The regions downstream and upstream of the OCCR were defined as the first nucleotide at the prime side up to the nucleotide before the start of the OCCR. Large rearrangements were classified in the according category based on their location.

Descriptive statistics were used to describe the study population and the carrier reference population. Only the first primary breast cancer was considered as an event and censoring was applied at the age of RRM (N = 177), RRSO (N = 342), ovarian cancer diagnosis (N = 298), last moment of contact (N = 914), or death (N = 33). To estimate the cumulative incidence of breast cancer in *BRCA1* and *BRCA2* carriers in both regions, crude (unadjusted) age-related breast cancer rates were calculated using Kaplan-Meier survival analyses, and the corresponding confidence intervals were calculated with a log-log approach based on Greenwood standard errors (24). To adjust for a possible birth cohort effect and mutation spectrum, normalized weights (one divided by the number of combination of the categories of the confounding variables) were calculated and applied in the Kaplan-Meier analyses. Through this weighing, the percentage of women in the different birth cohort categories (≤1920, 1921-1930, 1931-1940, 1941-1950, 1951-1960, 1961-1970 and ≥1971) and the mutation spectrum (5' to OCCR, OCCR, and 3' to OCCR) were identical in each category and also in the study and the carrier reference population, without affecting the total sample size.

Regional differences in the age-related breast cancer incidences in *BRCA1/2* carriers in the Northern Netherlands compared to the rest of the Netherlands were analyzed using Cox regression analyses. All hazard ratios were adjusted for birth year and mutation spectrum. To correct for ascertainment bias, these analyses were performed with and without the index cases. The assumption of proportionality was checked by assessing the log-minus-log plots and Schoenfeld residuals. The amount of confounding by the mutation spectrum was calculated as the change in the hazard ratio (25):

$$\frac{HR_{\text{adjusted for birth year}} - HR_{\text{adjusted for birth year and mutation spectrum}}}{HR_{\text{adjusted for birth year and mutation spectrum}}}$$

Differences in the background breast cancer risk in the general population in the UMCG catchment area compared to the rest of the Netherlands were tested using odds ratios. The cumulative incidence in the general population was calculated using the cumulative rates, which were calculated as the sum of the age-specific breast cancer incidence rates in the general population, multiplied by the width of the age-group (i.e. 5 years) (26).

All analyses were performed using SPSS version 20 (SPSS Inc., Chicago) and R version 3.0.1 (23), and statistical significance was defined as $p < 0.05$.

Results

Characteristics of study and carrier reference population

The study population consisted of 1,050 *BRCA* carriers: 656 *BRCA1* and 394 *BRCA2* (Table 1: data excluding index cases, Supplementary Table 1: data including index cases). These included 29% *BRCA1* and 32% *BRCA2* index cases. The carrier reference population from the rest of the Netherlands consisted of 1,580 *BRCA1* carriers, including 31% index cases, and 433 *BRCA2* carriers, including 30% index cases. The study population consisted of relatively more *BRCA2* carriers, but the family size of *BRCA* families was not different between the two populations. The average birth year in the study population was earlier in *BRCA1* carriers (1953 vs. 1955, $p = 0.025$) and *BRCA2* carriers (1952 vs. 1957, $p < 0.001$) compared to the carrier reference population.

In the study cohort including index cases, 46% *BRCA1* and 44% *BRCA2* carriers developed breast cancer. In the carrier reference population 45% *BRCA1* and 42% *BRCA2* carriers developed breast cancer (Supplementary Table 1). *BRCA1* and *BRCA2* carriers in the study population were diagnosed with breast cancer at an older mean age than carriers in the reference population, for *BRCA1* 42.4 (SD 10.1) versus 40.2 (SD 8.5) years ($p = 0.001$) and for *BRCA2* 47.1 (SD 10.6) versus 43.4 (SD 8.6) years ($p < 0.001$).

The mutation spectrum was known for all *BRCA1* and *BRCA2* carriers in the Northern Netherlands, and for 88% of *BRCA1* and 83% of *BRCA2* carriers in the rest of the Netherlands. In the Northern Netherlands, 39% *BRCA1* carriers had a mutation within the OCCR versus 23% carriers in the reference population ($p < 0.001$) (Fig. 1). For the *BRCA2* mutation in the Northern Netherlands, 18% mutations were within the OCCR versus 65% in the rest of the Netherlands ($p < 0.001$). This large difference in the mutation spectrum between *BRCA2* carriers in both populations was due mainly to the presence of three apparent northern founder mutations (c.9672dup, c.7419_7420delTG and c.1310_1313del: all three outside the OCCR).

Regional breast cancer risk estimates for carriers

For *BRCA1* carriers, the crude cumulative incidence of breast cancer by age 70 was 58% (95%CI 50-66%) in the Northern Netherlands, and 68% (95% CI 62-73%) in de rest of the Netherlands (Table 2). At 70 years of age, 24 (5%) and 28 (3%) women were still at risk of developing cancer in the *BRCA1* study and reference population, respectively

(Supplementary Table 2). When the index cases were included in both populations, these risks were 72% (95% CI 66-78%) and 76% (95% CI 71-79%), respectively. Weighted breast cancer risks that excluded the index cases and incorporated the differences in birth cohorts and mutation spectrum between both populations, resulted in lower risk estimates: 53% (95% CI 45-61%) in the Northern Netherlands and 55% (95% CI 47-63%) in the rest of the Netherlands (Table 2, Fig. 2, Supplementary Table 3).

In *BRCA2* carriers, the breast cancer risk at age 70 was 64% (95%CI 50-75%) in the Northern Netherlands and 61% (95% CI 50-69%) in the rest of the Netherlands. At 70 years of age, 11 (4%) and 14 (5%) women were still at risk of developing cancer, respectively. When the index cases were included, these risks were 78% (95% CI 69-85%) and 72% (95% CI 64-78%), respectively. For *BRCA2* carriers, the weighted breast cancer risk excluding the index cases resulted in higher estimates than the crude risks: by age 70 these were 66% (95% CI 54-78%) in the Northern Netherlands and 64% (95% CI 51-77%) in the rest of the Netherlands (Table 2).

Weighted cumulative incidence curves stratified by mutation spectrum and the related relative risks are presented in Fig. 3 and in Supplementary Table 4, respectively. The different categories of the mutation spectrum were related to different breast cancer risks, with lower breast cancer risk estimates for mutations within the OCCR. These differences were more outspoken in *BRCA2* carriers, and opposite effects (increased vs. decreased risks) were shown in the study population and reference population. No significant differences were revealed in regional comparisons of each specific mutation, but numbers were small (data not shown).

Regional breast cancer risks differences

BRCA1 carriers (excluding index cases) in the Northern Netherlands had a lower risk of developing breast cancer than those in the rest of the Netherlands, HR = 0.66 (95%CI 0.54-0.81) (Table 3: data excluding index cases, Supplementary Table 5: data including index cases). When only data from women with a known mutation spectrum were included, this HR was 0.73 (95%CI 0.59-0.89). The amount of confounding by the mutation spectrum was 3%. Correction for the mutation spectrum increased the HR with this amount, and resulted in a HR of 0.75 (95% CI 0.61-0.93).

For the incidence curves of the *BRCA2* populations, the assumption of proportionality in the Cox model was not met; the lines crossed at approximately age 60 (Fig. 2). Below age 60, the breast cancer risk of *BRCA2* carriers in the Northern Netherlands was lower than that in the rest of the Netherlands, both excluding index cases (HR = 0.64, 95% CI 0.51-0.81) and including index cases (HR = 0.62, 95% CI 0.49-0.78). Strikingly, correcting for the mutation spectrum reduced the HR by 32%, which resulted in a fifty percent lower breast cancer risk in the Northern Netherlands (HR = 0.50, (95% CI 0.33-

0.77). From age 60 onwards, however, the breast cancer risk of *BRCA2* carriers in the Northern Netherlands was at least twice as high: the HR was 3.99 (95% CI 1.11-14.35) excluding the index cases and 2.56 (95% CI 1.91-5.48) including the index cases. When we looked at only carriers with known mutation spectrum data, the hazard ratios decreased and became non-significant. Correcting for the mutation spectrum reduced the HR by 11% to a HR of 2.61 (95% CI 0.68-10.0).

Population background incidence

There appeared to be no significant differences in the breast cancer incidence in the general population between the Northern Netherlands and the rest of the Netherlands: the OR over the ages 20-75 was 1.03 (95%CI 0.97-1.09): under 60 1.02 (95% CI 0.94-1.11) and over 60 1.03 (95% CI 0.97-1.09). Neither were there birth-cohort related differences in the regional odds ratio, and the cumulative incidence curves were parallel over time (Fig. 2).

Discussion

We aimed to determine whether there were regional differences in the breast cancer risk in *BRCA1/2* carriers ascertained and analyzed in one identical way, and if so, whether they could be explained by the mutation spectrum or population background risk. We found that all *BRCA1* and the *BRCA2* carriers under age 60 had a significantly lower breast cancer risk in the Northern Netherlands, HR excluding index cases were 0.66 (95% CI 0.54-0.81) and 0.64 (95% CI 0.51-0.81), respectively. From age 60, the breast cancer risk in *BRCA2* carriers in the Northern Netherlands was higher than in the rest of the Netherlands (HR excluding index cases: 3.99, 95% CI 1.11-14.35). Adjustment for the mutation spectrum changed these HRs for *BRCA1*, *BRCA2*<60 and *BRCA2*≥60 by -3%, 32% and 11%, respectively. The background breast cancer incidence did not explain any risk difference: OR under 60 was 1.02 (95% CI 0.94-1.11) and OR over 60 was 1.03 (95% CI 0.97-1.09).

This study confirmed regional breast cancer risk differences, especially for *BRCA2* carriers. We estimated a cumulative incidence of breast cancer by age 70 excluding indexes, of 58% (95% CI 50-66%) for *BRCA1* carriers and 64% (95% CI 50-75%) for *BRCA2* carriers in the Northern Netherlands. For *BRCA1* carriers this was similar to the previous estimate, but the *BRCA2* risk decreased from 78% to 64% (6). The lower risk in the current *BRCA2* cohort can be explained by the fact that now more carriers (+9%) have a mutation within the OCCR and more carriers (+8%) are born in the ≥ 1971 cohort as compared to the previous study. Besides, to enable comparison with the national HEBON cohort data, we applied censoring for all RRSOs, whereas in the previous study, censoring was only applied when RRSO was performed before 50 years of age.

Published estimations of the breast cancer life time risk of *BRCA1/2* carriers show considerable variation: 40-87% for *BRCA1* and 28-88% for *BRCA2* carriers (2, 3, 7-9, 27). This variation can be explained by differences in population, in ascertainment and in methodology. Our current estimates of lifetime risks for the rest of the Netherlands are higher than those recently published in the same cohort (10): 68% (95% CI 62-73%) versus 45% (95% CI 36-52%) for *BRCA1*, and 61% (95% CI 50-69%) versus 27% (95% CI 14-38%) for *BRCA2* carriers. These low estimates of Brohet *et al* can be explained by inclusion of older birth cohorts (with lower breast cancer penetrance), and by the applied method (modified segregation analysis has a stronger correction for bias). When they included only cohorts from 1940 onwards, their estimates substantially increased: from 45% to 66% for *BRCA1* and 27% to 32% for *BRCA2* by age 65.Though based on mainly retrospective data, our breast cancer risk estimates of the carriers in the Netherlands are very similar to those published in a recent prospective study: 60% (95% CI 44-75%) for *BRCA1* and 55% (95% CI 41-70%) for *BRCA2* carriers by age 70 (27). However, in this prospective study the peak incidence among *BRCA2* carriers was in the age group 40-49 years, which is earlier than in the Netherlands.

We found that differences in the mutation spectrum between the Northern Netherlands and the rest of the Netherlands partly explained the regional breast cancer risk differences, which was seen in the adjusted hazard ratios and illustrated with weighted risk estimates. The magnitude of confounding by the mutation spectrum was largest (32%) among *BRCA2* carriers below 60, as compared to 3% and 11% in *BRCA1* and *BRCA2* carriers over age 60, respectively. Our analyses showed that *BRCA1* carriers and *BRCA2* carriers under age 60 that live in the Northern Netherlands have a lower breast cancer risk than those in the rest of the Netherlands: the HRs adjusted for birth year and OCCR were 0.75 and 0.50, respectively. Controversially, above age 60, *BRCA2* carriers in the Northern Netherlands had a higher, though statistically non-significant, breast cancer risk (adjusted HR=2.61, 95% CI 0.68-10.00). When the crude and adjusted *BRCA2* ≥60 model only included carriers with a known mutation spectrum, the risk difference was insignificant, which was probably due to a lack of power. The differences in the breast cancer risks between each gene region, and the different distribution of mutations between the Northern Netherlands and the rest of the Netherlands, underline the importance of the confounding effect of mutation spectrum. Strikingly, in *BRCA2* the HRs of the 3' versus the 5'region were significantly different in the opposite direction in both the study and the reference population. This might indicate that smaller defined regions on the *BRCA2* gene could explain breast cancer risk differences better, making the (previously found) regional differences in the frequency of specific *BRCA1* or *BRCA2* mutations in the Netherlands even more important (12).

We found that the breast cancer incidence of women in the general population did not differ between the Northern Netherlands and the rest of the Netherlands (OR=1.03, 95% CI 0.97-1.09), and this cannot therefore explain the regional

risk differences among *BRCA1/2* carriers. In a recent report from the Dutch Institute for Public Health and the Environment, it was shown that in one of the three northern provinces of the Netherlands, there was a small, but statistically significant, increased breast cancer risk of 1.06 compared to the national average risk, whereas for the other two provinces the 1.02 and 1.03 risk increase was non-significant (28).

The strengths of our study are the inclusion of the relevant target population of *BRCA1/2* carriers (i.e. carriers who receive the actual counseling), the large sample size, and the partly prospectively collected data. Our study gives a new perspective on the existence and explanation of regional differences in the breast cancer risk of *BRCA1/2* carriers, using a two-way approach involving genes (mutation spectrum) and environment (population background risk). Moreover, the study and the carrier reference population are family-clinic based populations, using the same national referral and DNA testing criteria and methodology, with well-documented information on carrier status and cancer development. Differences in the ascertainment between the study and reference carrier population could affect the results. However, comparison of indicators of the ascertainment of both populations showed that 76% of the tests were pre-symptomatic, and showed no statistically significant differences in proportion of (pre)symptomatic genetic tests, mean age at genetic test, proportion of test above age 60 and proportion of these tests per family. The population in the Northern-Netherlands was ascertained up to September 2011 and the Rest of the Netherlands up to 2006 ,but there was also no indication for testing bias when only carriers ascertained up to 2006 were assessed. This more recent period of ascertainment is a likely explanation for the higher uptake of preventive surgery in the northern region. Up to 2006, women were offered the choice between ovarian screening and RRSO. From 2007 onwards, counseling changed and ovarian screening was gradually abandoned because of proven ineffectiveness. Our center was one of the first centers to change this policy. As a result, women more often choose for RRSO and RRM, and also at an earlier age (29). The RRSOs were truly prophylactic since before RRSO all women underwent transvaginal ultrasonography and serum marker CA125 to exclude manifest ovarian cancer (30). Underreporting of preventive surgery in the rest of the Netherlands was very unlikely as medical files of proven carriers were available.

A limitation of our study is that the *BRCA1/2* carriers were ascertained through family cancer clinics, which may have resulted in an over-estimation of the breast cancer risks. Moreover, the study population included both incident and prevalent breast cancer cases, which also could have led to over-estimation. However, the over-estimation is negligible, since the follow-up time of known carriers and former untested relatives (N=273) was extended with 61 months (SD 40), and included 19 new primary breast cancers, which is only 6% of all cases.

Besides, the percentage of ovarian cancer and preventive surgery was higher in the study population as compared to the reference population. This may have led to a higher percentage of censored cases in the study population. Therefore, the

breast cancer risk could be underestimated in the study population as compared to the carrier reference population, because after these censoring events some carriers will develop breast cancer (31). Since the medical files were available, underestimation of the risk due to missing data on breast cancer, ovarian cancer or preventive surgery is unlikely. For the rest of the Netherlands, missing data for the age at cancer diagnosis in the pedigree was imputed based on birth cohort specific population data (10). However, it is unknown whether this was necessary for the carriers included in this study (born after 1910). Future linking to national pathology and cancer registries will help to indentify and quantify missing data.

In conclusion, *BRCA1/2* carriers in the Northern Netherlands were at lower risk of developing breast cancer than in the rest of the Netherlands, except for *BRCA2* carriers over age 60, whose risk was doubled. Differences in the mutation spectrum between the two regions partly explain the risk differences seen in *BRCA1/2* carriers, with a somewhat stronger effect in *BRCA2* carriers. Both for *BRCA1* and *BRCA2* carriers, the detected regional risk differences could not be explained by differences in the background cancer risk in their respective general populations. Possible other explanations for the regional risk differences could be differences in (founder) mutations in modifier genes, lifestyle and/or gene-environment interactions. Especially, a large scale international study on the breast cancer risk and mutation position in *BRCA* carriers can be worthwhile due to the limited sample sizes in this study. The increased risk in *BRCA2* carriers over age 60 in the Northern Netherlands should be a good reason to consider extending intensive breast cancer screening to *BRCA2* carriers beyond the age of 60 in this region.

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Table 1 Clinical characteristics of *BRCA1/2* carriers excluding index cases in the Northern Netherlands (study population) and in the rest of the Netherlands (reference population)

Characteristics	***BRCA1* carriers**			***BRCA2* carriers**		
	Northern Netherlands N = 467	Rest of the Netherlands N = 1091	*p*-value	Northern Netherlands N = 269	Rest of the Netherlands N = 305	*p*-value
Age at last follow-up, mean (SD)	47.6 (13.8)	42.1 (11.9)	<0.001	48.2 (14.1)	44.9 (11.9)	0.003
Primary breast cancer						
N (%)	157 (34%)	373 (34%)	0.861	74 (28%)	95 (31%)	0.359
Age, mean (SD)	43.6 (9.9)	41.2 (9.0)	0.006	49.1 (9.8)	44.5 (8.1)	0.001
At age 60, N (% of all breast cancers)	13 (8%)	12 (3%)	0.022	13 (18%)	3 (3%)	0.002
Ovarian cancer						
N (%)	75 (16%)	80 (7%)	<0.001	16 (6%)	21 (7%)	0.735
Age, mean (SD)	51.7 (10.0)	51.6 (9.7)	0.972	56.1 (9.6)	55.1 (11.5)	0.799
RRM						
Number (%)	86 (19%)	173 (16%)	0.207	36 (14%)	38 (13%)	0.709
Age, mean (SD)	40.9 (9.7)	39.9 (10.0)	0.486	41.6 (8.8)	41.0 (7.3)	0.740
RRSO						
N (%)	146 (31%)	227 (21%)	<0.001	83 (31%)	49 (16%)	<0.001
Age, mean (SD)	44.1 (8.5)	44.2 (8.4)	0.791	47.7 (9.6)	46.0 (8.8)	0.326
Pre-symptomatic BC at DNA test [a]						
N (% of all tests)	252 (78%)	519 (73%)	0.093	180 (83%)	113 (75%)	0.114
Over age 60, N (% of pre-symptomatic test)	23 (9%)	55 (11%)	0.528	19 (10%)	15 (13%)	0.574
Age, mean (SD)	40.3 (13.0)	40.6 (14.0)	0.792	42.2 (13.6)	43.1 (14.1)	0.582

Abbreviations: RRM: risk-reducing mastectomy; RRSO: risk-reducing salpingo-oophorectomy; BC: breast cancer

[a] Data on the demographic details of genetic testing were available for 74% of the populations

Table 2 Cumulative incidence of breast cancer in *BRCA1/2* carriers, excluding index cases, in the study population (Northern Netherlands) and reference population (rest of the Netherlands). Estimation of (A) the crude risk and (B) risk weighted to an equal contribution from the different birth cohorts and OCCR regions in each population.

Age (years)	***BRCA1* carriers**		***BRCA2* carriers**	
	Northern Netherlands	Rest of the Netherlands	Northern Netherlands	Rest of the Netherlands
(A) Crude risks	N = 467	N = 1091	N = 269	N = 305
≤30	2.8 (1.5-4.7)	3.7 (2.6-5.0)	0.4 (0.0-2.2)	0.7 (0.1-2.3)
≤40	16.0 (12.4-20.0)	23.1 (20.2-26.1)	7.3 (4.3-11.4)	13.3 (9.3-17.9)
≤50	37.8 (31.9-43.6)	46.6 (42.5-50.6)	28.2 (21.3-35.6)	37.6 (30.4-44.7)
≤60	50.1 (43.2-56.7)	61.1 (56.0-65.7)	46.3 (36.5-55.6)	54.8 (45.9-62.9)
≤70	58.4 (50.2-65.7)	67.9 (61.7-73.2)	63.9 (50.3-74.7)	60.6 (50.2-69.4)
≤75	61.2 (51.5-69.4)	71.0 (63.8-77.1)	68.4 (52.7-79.8)	-
(B) Weighted risks	N = 467	N = 913	N = 269	N = 229
≤30	1.9 (0.8-4.4)	2.7 (1.4-5.4)	0.3 (0.0-6.4)	0.2(0.0-2.0)
≤40	11.8 (8.4-16.4)	17.0 (12.9-22.2)	7.6 (4.1-13.8)	9.5 (4.8-18.2)
≤50	32.3 (26.4-39.0)	36.0 (30.0-42.7)	29.0 (21.3-38.6)	29.9 (20.6-42.1)
≤60	46.8 (39.8-54.4)	49.9 (42.7-57.5)	46.8 (36.8-58.1)	49.1 (37.5-62.1)
≤70	52.8 (45.1-61.0)	54.6 (46.9-62.7)	66.2 (54.2-77.7)	63.7 (50.6-76.6)
≤75	54.2 (46.2-62.7)	58.6 (50.2-67.2)	68.2 (55.8-80.0)	-

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Table 3 Regional differences in the breast cancer risk of *BRCA1/2* carriers excluding index cases (adjusted for birth year) in the Netherlands: the study population (Northern Netherlands) compared to the reference population (the rest of the Netherlands)

Model	**Regional differences**					
	BRCA1		*BRCA2* <60		*BRCA2* ≥60	
	N (events)	HR (95%CI)	N (events)	HR (95%CI)	N (events)	HR (95%CI)
Region						
Reference population	1091 (371)	1	305 (91)	1	29 (3)	1
Study population	467 (141)	0.66 (0.54-0.81)	269 (59)	0.64 (0.51-0.81)	38 (11)	3.99 (1.11-14.35)
Region						
Reference population	913 (281)	1	224 (56)	1	20 (3)	1
Study population	467 (141)	0.73 (0.59-0.89)	269 (59)	0.66 (0.46-0.96)	38 (11)	2.92 (0.81-10.55)
Region [a] corrected for OCCR						
Reference population	913 (281)	1	224 (56)	1	20 (3)	1
Study population	467 (141)	0.75 (0.61-0.93)	269 (59)	0.50 (0.33-0.77)	38 (11)	2.61 (0.68-10.00)

[a] Including only carriers with known mutation spectrum

Fig. 1 Overview of mutation spectrum in the study population (Northern Netherlands) and reference population (rest of the Netherlands): 3'to OCCR (3'), within OCCR (OCCR) and 5' to OCCR (5').

Fig. 2 Cumulative incidence of breast cancer in *BRCA1/2* carriers, excluding index cases, and the general population in the study population (Northern Netherlands) and reference population (rest of the Netherlands), with the carriers' risk weighted to an equal contribution from the different birth cohorts and mutation spectrum in each population

Fig. 3 Cumulative incidence of breast cancer in *BRCA1* (A) and *BRCA2* (B) carriers, excluding index cases, in the Northern Netherlands (study population) and rest of the Netherlands (reference population), with risk weighted to an equal contribution from the different birth cohorts in each population.

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Figure 1

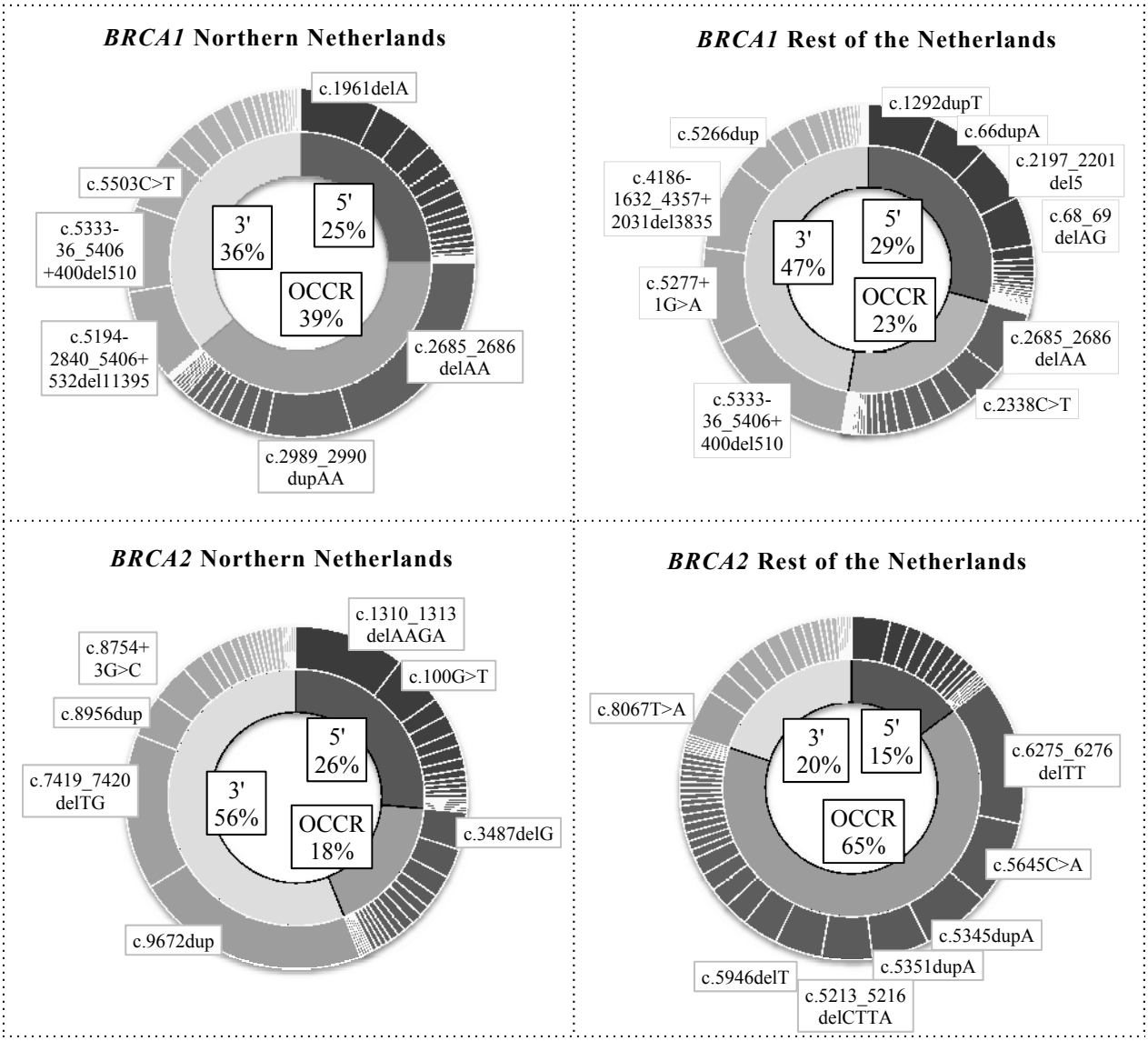
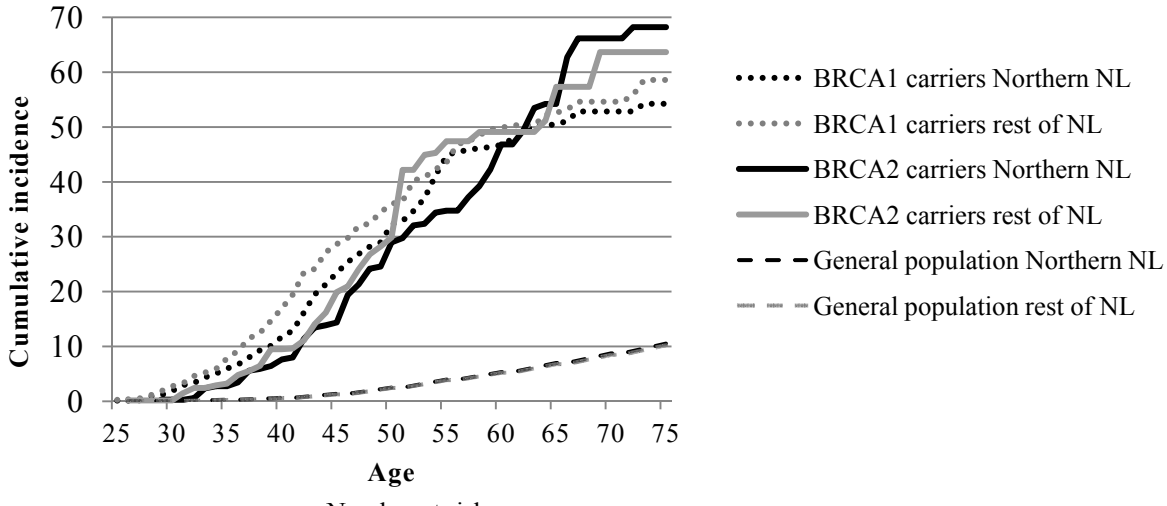


Figure 2

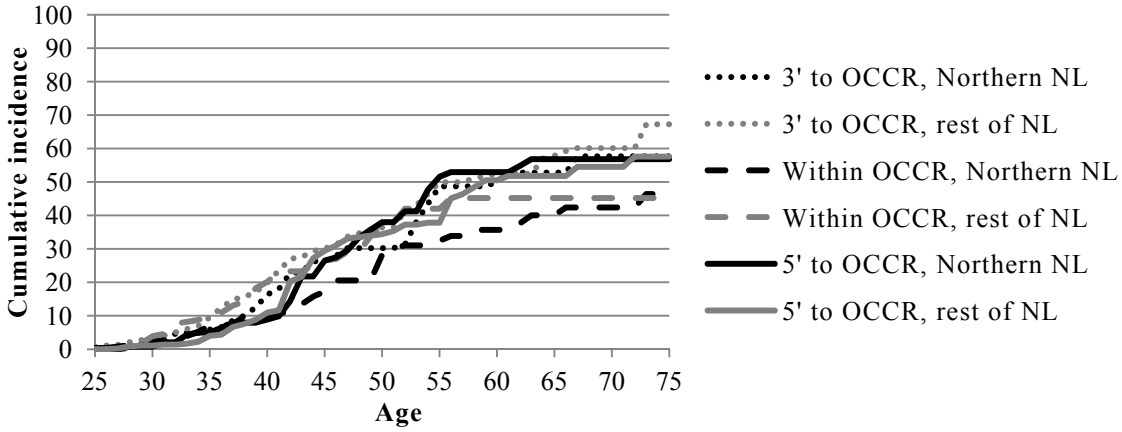


Number at risk

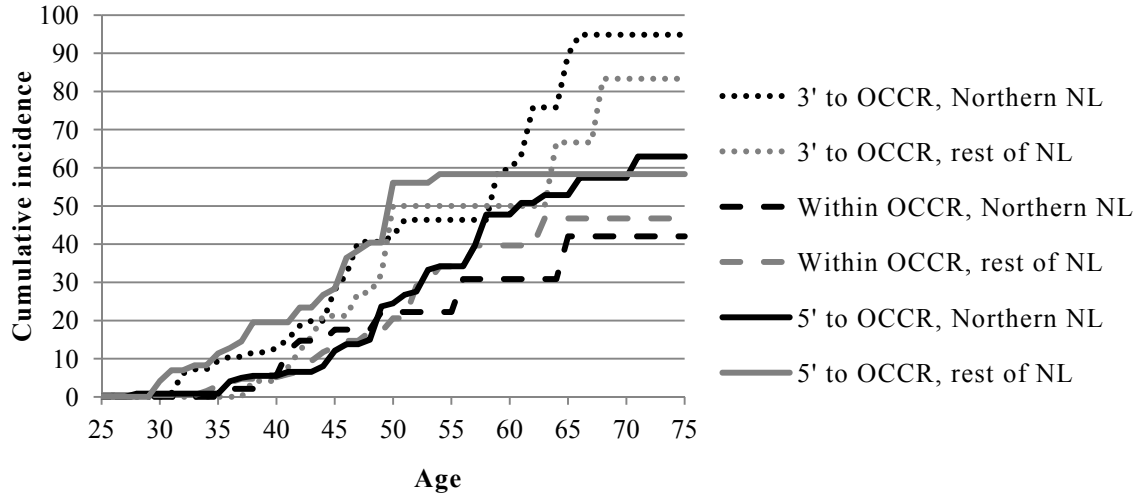
30	40	50	60	70	
330	301	231	137	79	*BRCA1* carriers Northern Netherlands
458	402	287	170	87	*BRCA1* carriers rest of the Netherlands
179	166	136	90	44	*BRCA2* carriers Northern Netherlands
190	168	124	82	43	*BRCA2* carriers rest of the Netherlands

Figure 3

A. BRCA1



B. BRCA2



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