Cancer risk variation in BRCA1/2 mutation families
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Chapter 8

General discussion
Summary

The (lifetime) risk of cancer in BRCA1/2 mutation families has been studied extensively, although results show a wide variation, which is confusing for clinical practice. The aim of this thesis was to improve the accuracy of the breast and ovarian cancer risk estimates in BRCA1/2 mutation families by understanding the reasons for risk variation and assessing the effect of suggested risk factors, especially familial factors, more closely. Both absolute and relative breast and ovarian cancer risks were assessed in mutation negative and mutation positive women in BRCA1/2 mutation families. Moreover, the implications of the CLTRs for breast cancer screening above age 60 in BRCA1/2 mutation carriers were evaluated.

Cancer in proven non-carriers
For proven non-carriers in BRCA1/2 mutation families, study results are contradicting on whether or not these non-carrier relatives are at increased risk for breast cancer as compared to women in the general population, whereas for ovarian cancer no risk estimates for non-carriers were available at all. Both breast and ovarian cancer risks of proven non-carriers in BRCA1/2 mutation families were assessed in a consecutive cohort counseled at the Family Cancer Clinic in the Northern region of the Netherlands (chapter 2). The results showed that the lifetime risk of breast cancer was slightly increased (SIR 1.5, 95%CI 0.9-2.3) as compared to the general population, whereas for ovarian cancer no risk increase was detected, though these numbers were small. The breast cancer risk increase was more pronounced in non-carriers from BRCA1 mutation families (SIR 2.0, 95%CI 1.1-3.3), especially in age-group 40-49 (SIR 4.5, 95%CI 1.8-9.2). At first sight the stronger risk increase in the 5th decade might be considered as a sign of ascertainment and genetic testing bias, however, the effect remained when bias correction was performed through inclusion of a proportion of untested FDRs. Only among BRCA1 mutation families a difference in familial load (i.e. a more suspected family history) was observed between families with affected non-carriers before age 50 and the rest of the families.

Breast cancer risk variation in the Netherlands
Previously relatively high breast cancer risks, especially at older age, were observed for mutation carriers in the Northern-Netherlands (up to 88% for BRCA2) as compared to carriers in the rest of the Netherlands (Geo-Hebon cohort). In order to assess whether mutation carriers in the Northern-Netherlands are indeed at higher risk as compared to the rest of the Netherlands and to disentangle this effect from the impact of differences in study methodology and bias, we examined and quantified the existence of regional risk differences in the Netherlands by using these two Dutch clinic-based cohorts and analyzing them identically (chapter 3). BRCA1 mutation carriers in the rest of the Netherlands were at a somewhat increased risk (HR = 1.52, 95%CI 1.23-1.85) as compared to the Northern-Netherlands as were the BRCA2 mutation carriers up to age 60 (HR = 1.56, 95%CI 1.04-2.17). From age 60 onwards BRCA2 mutation carriers in the Northern-Netherlands were at increased risk when compared to BRCA2 carriers.
in the rest of the Netherlands (HR = 3.99, 95% CI 1.11-14.4). Regional differences in the mutation spectrum exist, especially for \textit{BRCA2} due to several Northern founder mutations, but these only partly explained the observed regional risk differences.

\textbf{Breast cancer risk variation explained by methodology}

For \textit{BRCA1/2} mutation carriers, the breast cancer lifetime risk estimations vary from 27\% to 88\%. The regional risk differences in the Netherlands appeared smaller than what could have been expected as based on the CLTRs for the separate cohorts (chapter 3). This was suggestive of a possible impact of differences in study methodology. The impact of risk assessment methodology and bias correction was explicitly assessed by applying all applicable methods -in total 19- to one well-defined clinic-based cohort (chapter 4). These different methods consisted of Kaplan-Meier analyses with and without bootstrapping, of frailty model analyses and of modified segregation analyses for risk estimation, in combination with different forms of bias correction, such as excluding indexes, including untested FDRs or conditioning the likelihood function on phenotype and genotype. The estimates of all methods resulted in a variation of the CLTRs of 35\% to 83\% for \textit{BRCA1} and 41\% to 86\% in \textit{BRCA2} mutation carriers. Much of the variation seen in the CLTRs could be explained by the different bias correction methods, and not so much by population differences. Results of our simulation study showed that the Kaplan-Meier estimation with bias correction through inclusion of a proportion of untested FDRs was the most suitable method for risk estimation in \textit{BRCA1/2} mutation carriers counseled in the Family Cancer Clinic. The modified segregation analyses with conditioning of the likelihood function on index cases' genotype and all phenotypes provided risk estimates more suitable for population-based cohort of \textit{BRCA1/2} carriers.

\textbf{Inverse trends in ovarian cancer risk}

For breast cancer the CLTR increases in more recent birth cohorts both in the general and in the \textit{BRCA1/2} carrier population. This is most likely due to a combination of changes in lifestyle, hormonal and reproductive factors. The results of previous studies on the effect of the birth cohort on the ovarian cancer risk in \textit{BRCA1/2} mutation carriers also indicated an increase but were inconsistent. However, this possible risk increase is in contrast with the decreasing ovarian cancer incidence by birth cohort observed in the general population. In a regional clinic-based cohort of mutation carriers and their background general population it was assessed whether a birth cohort effect existed for ovarian cancer as this effect is known to be present for breast cancer (chapter 5). Our results show that the ovarian cancer in \textit{BRCA1/2} mutation carriers is increased in more recent birth cohorts, even though the risk is decreasing in their background general population in more recent cohorts. \textit{BRCA1/2} mutation carriers born more recently, particularly those with a \textit{BRCA1} mutation, have a higher additional ovarian cancer risk. The reason for this is not yet clear.
The parent-of-origin effect for breast cancer risks
It has been suggested that the effect of the parent-of-origin of the \textit{BRCA1/2} mutation is associated with the risk of breast cancer. The presence of the parent-of-origin effect, and whether this effect is independent from referral bias due to differences in the family or personal history of cancer was assessed using the national clinic-based Hebon cohort (chapter 6). The results showed that the presence of a parent-of-origin effect depends on the way one corrects for referral bias. Correction of referral bias as defined by family history did not substantially impact this effect, while bias correction for the personal cancer history made the parent-of-origin effect disappear. This bias when uncorrected, may have caused the positive association between paternal origin of the \textit{BRCA1/2} mutation and risk of breast cancer in earlier studies. In a larger prospective cohort the impact of referral bias by a combined assessment of family and personal history should be addressed.

Breast cancer screening from age 60 onwards
In the Netherlands, \textit{BRCA1/2} mutation carriers are offered intensive breast cancer screening from age 25 to age 60. Above age 60, they are offered less intensive screening till age 75 consisting of either annual mammography or the national screening program with biennial mammography. The relevancy and efficacy of screening mutation carriers aged 60 year and over was assessed using the cohorts of the Family Cancer Clinic in Rotterdam and Groningen and the national Hebon cohort (chapter 7). More than 70% of the mutation carriers over age 60 still has one or two breasts, and therefore remains at risk for breast cancer. More than half of the tumors detected by biennial mammography were of unfavorable stage, which was more than two times higher as by annual mammography. Above age 60, \textit{BRCA1/2} mutation carriers are still at risk for breast cancer and annual mammography might be beneficial.

Methodological considerations
In research, study designs and biases are often a topic of debate, and assumptions regarding the impact or existence of these methodological issues are made on a regular basis. As these methodological issues can influence the outcome of the study, it is important to stress the following methodological considerations. For accurate risk estimations several methodological issues regarding statistics, bias correction as well as population and familial factors are important to consider. Which risks are we estimating, and for whom are we estimating them? Is the study cohort the proper cohort for the analyses? How are the subjects ascertained and are there causes for bias? When does the follow-up time start, and when was the DNA test performed? Which events are being counted and at which events should censoring be applied? And, when comparing groups or estimating relative risks, are there demographic differences between the groups or confounding factors that should be taken into account?
Target population: is the study population suitable to answer your research question?
When estimating CLTRs it is important to keep the target population in mind: for which population will the calculated cancer risks be applicable? For BRCA1/2 carriers that are referred to the familial cancer clinics, or for unselected carriers from the general population? For the estimation of breast cancer risk for BRCA1/2 mutation carriers both clinic-based and population-based study cohorts have been used. However, it is for the women seen in the Family Cancer Clinic, i.e. those women who receive the actual counseling, that we need adequate risk figures the most. Of all BRCA1/2 families, only the high-risk BRCA1/2 families will enter the clinic since their personal and/or family history meets the national referral criteria. Even when the risk estimates are based on a clinic-based population, just using every female mutation carrier for risk estimation would result in incorrect risk estimates as this population is enriched with cancer cases (e.g. early onset and/or bilateral breast cancer, or ovarian cancer), because they were the ones referred as index cases. So when assessing absolute or relative risks, the ascertainment bias (also called selection bias or referral bias) and genetic testing bias -meaning that affected women are more likely to undergo DNA testing- should be addressed.

Prospective versus retrospective cohorts
Most studies on cancer risks in BRCA1/2 mutation families -including the ones in this thesis- are based on retrospective or consecutive cohorts. However, it is generally assumed that prospective studies are preferable, because reported information is more accurate and the design can already take the above described biases into account. Inclusion of all cancer cases, including those cases that led to the ascertainment of the family because of referral to the Family Cancer Clinic, might be troublesome when assessing the effect of family history factors, since there is overlap between the selection criteria (familial cancer as reason for referral), the risk predictors (familial cancer load as separate risk factor) and possibly the outcome event (cancer as event and/or reason for censoring). However, in a prospective analysis -including only incident cases that occurred after a person’s date of genetic testing- the follow-up time, especially at older age, and the number of incident cases are still rather limited, because 1) genetic testing for BRCA1/2 mutations started small scale (only) 20 years ago, and 2) the percentage of women opting for risk-reducing surgery is increasing, which markedly reduces the collection of follow-up data. An alternative approach could be to only include follow-up time and events that occurred after the first BRCA1/2 mutation has been found in the family (i.e. the index), but here genetic-testing bias is still in play. However, the follow-up time at older age remains an important factor with respect to the certainty and applicability of the risk estimates.

Familial clustering
There are two reasons for incorporating the family structure or familial clustering in the analyses: dependent sampling and unobserved heterogeneity. First, in BRCA1/2 mutation families the sampling of the family members does not occur independently as it occurs via a cascade screening approach within the family. This implies that mutation carriers are not independent from each other but correlated, while independent or
random sampling is the statistical assumption. When clustering is presented but not incorporated in the model the standard errors will be smaller than they should be. Second, \textit{BRCA1/2} mutation families seen in the Familial Cancer Clinic setting all meet the referral criteria, but this referred \textit{BRCA1/2} population is not homogeneous. Some families have a stronger family history than others, and in some families a history of breast cancer predominates while in others a history of ovarian cancer is most outspoken. This familial heterogeneity can be a chance phenomenon, but it can also be due to differences in the underlying risk profile consisting of the \textit{BRCA1/2} mutation type and other genetic and non-genetic factors that family members share and that lead to different phenotypic expression. For example, two sisters (i.e. 1\textsuperscript{st} degree relatives) share more (non-) genetic factors than two women from two different families, but they share also more than two cousins (i.e. 3\textsuperscript{rd} degree relatives) in one family. Because not all factors contributing to the cancer risks are known or measured, there is so called unobserved heterogeneity. For either of the two reasons to take the familial clustering into account, the familial clustering should be considered also when one is interested in marginal (i.e. population-averaged) estimates and the familial clustering is considered to be a nuisance.

For the study results in this thesis, the estimated clustering or heterogeneity between families was small and incorporating the familial clustering had only a small impact on the standard errors (i.e. larger standard errors) of the marginal estimates. However, this could have been because the definition of a family was rather broad and included besides close relatives also more remote relatives. Other studies have shown a larger impact of familial clustering when only first- or second-degree relatives were grouped in a family cluster.\textsuperscript{1}

\textbf{Confounding factors}

In (epidemiological) research it is important to consider any type of bias, including bias by confounding factors, i.e. factors related to both the outcome and predictor. When assessing relative risks of cancer in observational, non-matched cohorts, confounding factors might distort part of the effect seen. For example, in the assessment of the regional risk difference in the Netherlands (this thesis), the two regional cohorts had a different distribution of the \textit{BRCA} mutation spectrum. The mutation spectrum was related to the region but is also known to be related to the breast cancer risk, and was therefore included a confounding factor. Sometimes the relevance of a confounding factor is assessed by how much the outcome of interest changes when the confounder is taken into account. When this change is more than a pre-defined percentage (e.g. 5\%) the factor is considered relevant and remains included in the analysis.
Implications for practice and research

Understanding the variation in calculated cancer risks is important for comparing risk estimates in the literature, for re-evaluation of guidelines for counseling of population-averaged risk estimates, as well as for the development of more patient-tailored risk estimates. BRCA1/2 mutation carriers face complicated choices based on a fixed range of breast and ovarian cancer lifetime risks. Although a patient’s decision-making regarding preventive options is influenced by a complex network of factors, tailored cancer risk estimation could be beneficial as it could reduce part of uncertainty and might help to choose the right thing to do, at the right time.2, 3

The research in this thesis has detected several risk associations and trends that could contribute to the development of more personalized risk estimates or that are relevant for other aspects of clinical practice. For some of the observed effects the underlying cause is still unexplained and this should be addressed in follow-up research. The implications for clinical practice and follow-up research will be discussed here: starting with breast and ovarian cancer risks in mutation carriers, followed by breast cancer screening in mutation carriers, and ending with cancer risks in non-carriers in mutation families.

Breast cancer risk counselling in mutation carriers

Referral criteria have changed over time and have become slightly less stringent. Since less than 2 years, ovarian cancer patients can be referred to the Family Cancer Clinic irrespective of their family history, because family history was not a good predictor for mutation carriership.4 Also for breast cancer patients such a trend may develop, the age cut-off for referral of breast cancer patients with triple negative cancer (50 years) is already a topic of discussion. These changes in referral criteria mean that in the (near) future the clinic-based population of mutation carriers will no longer consist of only the selected high-risk population. The currently counselled breast and ovarian risk estimates might not be applicable to all mutation carriers, as (in this thesis) it is shown that different breast cancer risk estimates apply to selected mutation carriers (clinic-based cohorts) and unselected mutation carriers (population-based cohorts). More tailored risk counselling is one way to deal with this expected growing heterogeneity among BRCA1/2 mutation families. In this thesis it is shown that risk estimates probably can be refined using birth cohort, mutation spectrum and family history. Risk tailoring based on these confirmed and rather easy to measure risk factors would be a good step in the direction of more personalized risk counselling. In the meantime continuing research should focus on the elucidation of this heterogeneity by assessing SNPs, environmental factors and the interaction between the genetic and non-genetic factors. As for such studies enormous sample sizes are needed, continuation of (inter-) national registration of mutation families and cancer cases is crucial.

Irrespective of the discussion on tailoring of cancer risks, improvements can be made to current counselling of population-averaged risks by incorporating residual risks or 10-year risks, and expanding lifetime risks data till age 80 or over. Counselling of only lifetime risks might not always be appropriate, and residual cancer risks
might be more informative. For example, for a 55-year old mutation carrier, what is her risk as she already survived so many years cancer-free? As the residual cancer risks are based on the curve of the lifetime risk, these estimates can be determined using suitable lifetime risk curves. However, most studies provide risk curves and risk estimations up to age 70, while the average life expectancy for women is more than 10 years higher and still increasing. Some risk assessment tools, e.g. Boadicea, do provide risk estimates up to age 80, but these estimates are not yet based on the Dutch population.

Breast cancer screening from age 60 onwards.
The breast cancer risk in mutation carriers is still increasing after age 60. BRCA2 mutation carriers in the Northern-Netherlands over age 60 had a more increased breast cancer risk as compared to those in the rest of the Netherlands. It should be assessed whether, besides the BRCA1/2 mutation, other genetic and non-genetic factors can explain this lasting risk increase. Understanding this risk difference might give indications regarding which women might benefit most from breast cancer screening above the age of 60. Offering annual mammography to BRCA1/2 mutation carriers may be beneficial to detect tumors in a more favorable stage. Over time, guidelines have changed on this topic and mutation carriers over age 60 with dense breasts are already offered annual mammography outside the national screening program. It could be considered to offer this to all mutation carriers over age 60 with a good life expectancy.

Ovarian cancer risk in mutation carriers
For ovarian cancer, a similar discussion on risk tailoring and improvement of population-averaged applies. Even more so, because for ovarian cancer no screening is available and risk-reducing surgery is the only way for cancer prevention. This surgery –when performed in the appropriate time window- leads to immediate and early menopause, which may lead to short-term and long-term morbidity. Therefore, more personal or age-related cancer risk estimates are necessary for counselling on the optimal timing of RRSO.

For ovarian cancer also varying population-averaged risk estimates are published. An assessment of the effect of different methods of risk estimation and bias correction might be insightful, especially as for ovarian cancer the general population risk and population selection might show more variation among studies and countries. For example, in the general population of the Netherlands the incidence of ovarian cancer is declining over time, while this is not the case for all countries. The decreasing trends in the Netherlands is most probably due to the wide spread use of oral contraceptives.

For risk tailoring it is important to understand the birth cohort effect and the conflicting trends in the ovarian cancer risk in BRCA1/2 mutation carriers and general population. A starting point for this understanding might be to explore possible differences in oral contraceptive use between the two populations.

Proven non-carriers
For proven non-carriers in BRCA1/2 mutation families an increased risk was observed in the 5th decade. Annual mammography from age 40 to 49 may be considered, if the
results are confirmed in a upcoming national study. However, it could be that only a subgroup of non-carriers is at increased risk, due to underlying (non-) genetic risk factors. In this thesis the increased risk was mainly observed in non-carriers in \textit{BRCA1} mutation families, while in a recent study from the UK an increased risk was observed among non-carriers in \textit{BRCA1/2} mutation families,\textsuperscript{13} whereas in their prospective analyses the risk increase was mainly present in non-carriers in \textit{BRCA2} mutation families.

To improve the age-related risk counselling of proven non-carriers, follow-up research on the genetic load using OncoPanels, and the effect of SNPs and environmental factors (e.g. BMI, use of oral contraceptives, reproductive history and breast density) should be conducted to unravel the factors driving the increased cancer risk in non-carriers. Depending on the outcomes, personalized breast cancer screening might be explored for proven non-carriers.\textsuperscript{14-16}