General discussion

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1. Introduction

Women carrying a germline mutation in the breast cancer genes *BRCA1* and *BRCA2* have substantially increased risks of breast and ovarian cancer \(^{3,4}\). However, many gene carriers do not develop the diseases, even into old age, suggesting that the risks are modified by genetic and/or environmental risk factors \(^{5-12}\). This aims to estimate cancer risks in Dutch *BRCA1/2* families and to examine the variation in risks found within and between families. This was only possible through a nationwide collaboration between all Dutch Clinical Genetic Centers or the Hereditary Breast and Ovarian Cancer study, Netherlands (HEBON). Next, we used the International *BRCA1* and *BRCA2* Carrier Cohort Study (IBCCS) to investigate hormonal factors as risk modifiers for the development of breast cancer in *BRCA1* and *BRCA2* mutation carriers. In Section 2 of the general discussion the main research findings of this thesis will be described. Section 3 describes the methodological considerations regarding the study population, the penetrance estimates and the strengths and limitations of the studies. Section 4 puts the results in perspective and describes the potential role of *BRCA1* in estrogen-induced breast cancer followed by Sections 5 and 6, in which clinical implications and recommendations for future research are given.

2. Main research findings

Chapter 2 describes the estimation of penetrance of breast and ovarian cancer in *BRCA1/2* families. To estimate the age-specific relative risks (RR) of breast and ovarian cancers for each breast cancer gene, the conditional maximum likelihood approach was used. The heterogeneity of breast and ovarian cancer risk was examined by birth cohort, family history and mutation position. In total, 582 *BRCA1* and 176 *BRCA2* families were ascertained from eight Clinical Genetic Centres. The average cumulative breast cancer risk by age 70 years was estimated to be 45% (95% Confidence Interval (CI) =36-52%) for *BRCA1* and 27% (95%CI=14-38%) for *BRCA2* mutation carriers from these families. The corresponding cumulative risks for ovarian cancer were 31% (95%CI=17-43%) for *BRCA1* and 6% (95%CI=1-11%) for *BRCA2* mutation carriers. In *BRCA1* families the relative risks of breast and ovarian cancer increased with more recent dates of birth (RR=2.6, 95%CI=1.4-4.9) for breast cancer and for ovarian cancer (RR=3.7, 95%CI=0.9-15.8) for carriers born after 1940 compared to carriers born before 1940 \((P_{\text{heterogeneity}}=0.006)\). In *BRCA1* families the relative risk of breast cancer also increased with a stronger family history of breast cancer \((P_{\text{heterogeneity}}<0.001)\). Furthermore, for *BRCA1* a significant association between the location of the mutation and the ratio of breast to ovarian cancers was found \((P_{\text{heterogeneity}}<0.001)\). In contrast, in *BRCA2* families there was no evidence for risk heterogeneity by birth cohort, family history or mutation location, but power was limited for these comparisons due to a smaller sample size of families. In conclusion, the estimated breast and ovarian cancer risks of *BRCA1* and *BRCA2* mutation carriers were overall lower than the risks reported for other countries. The lower estimates for *BRCA1* might be due to the presence of older birth cohorts in our study population, a moderate family history, or specific founder mutations.

Chapter 3 describes the risk of cancers other than breast and ovarian cancer in 517 *BRCA1* families using a selected kin-cohort of family members with a 50% prior probability of being a carrier (N=6,585). Relative risks were calculated by observing the cancer incidences in this cohort with the expected site-, sex-, and period-specific population incidence rates. In total, 667 cancers at sites other than breast and ovary were observed (RR=1.04, 95% CI=0.97-1.12), with histological verification available for 43% of these tumors. *BRCA1* mutation carriers were at increased risks for cervical (RR=4.1, 95%CI=2.77-6.68), pharyngeal (RR=3.56, 95%CI=1.54-7.02), endometrial (RR=2.77, 95%CI=1.83-4.04), stomach (RR=2.59, 95%CI=2.01-3.28) and colon cancer (RR=2.51, 95%CI=2.02-3.07). Colorectal cancer (RR=1.29, 95%CI=1.05-1.57) appeared to be increased in women only. There was no risk increase of prostate and pancreatic cancer. Reduced risks were found for several sites, including rectal (RR=0.22, 95%CI=0.10-0.45) and lung cancer (RR=0.83, 95%CI=0.69-1.00), melanoma (RR=0.47, 95%CI=0.21-0.89) and kidney cancer (RR=0.26, 95%CI=0.13-0.47). This study confirms increased risks of cervical cancer and endometrial and possibly stomach cancer in *BRCA1* mutation carriers. At an increased risk for colorectal cancer was only observed in women, elevated risks may be due to misreporting of ovarian cancer. In contrast to some previous studies, no increased risks were observed for prostate cancer at a young age or pancreatic cancer.

In Chapter 4 cancer risks for sites other than breast and ovary were estimated in 139 *BRCA2* families using the same method as described above, with histological verification available for 46% of these tumors. Excess risks for four cancer sites were observed, i.e., pancreas (RR=5.9, 95%CI=3.2-10.0), prostate (RR=2.7, 95%CI=1.74-1.1), bone (RR=14.4, 95%CI=2.9-42.1) and pharynx (RR=7.3, 95%CI=2.0-18.6). Nearly all increased risks reached statistical significance for males only. Cancer risks tended to be larger when analyses were restricted to carriers below the age of 65 years. Moreover, families with mutations outside the previously defined ovarian cancer cluster region (OCCR) tended to have a higher risk of cancers at sites other than breast and ovary. Our study confirms that *BRCA2* mutation carriers are at increased risk for cancers of the prostate and pancreas, and possibly bone and pharynx. Knowing the risks for these cancers is clinically important for *BRCA1/2* mutation carriers, since risk reducing strategies and screening practices may be relevant for these cancer types. Therefore, further studies with more confirmed cancers are needed to elucidate the role of other cancers in *BRCA1/2* mutation carriers. The results of Chapters 3 and 4 provide further evidence that *BRCA1* and *BRCA2* have a distinct phenotype. The results indicated that *BRCA1* mutations are associated with gynaecological cancers, whereas prostate and pancreatic cancer appear to be part of the *BRCA2* phenotype. Premenopausal oophorectomy has been shown to substantially reduce the risk of ovarian cancer, but also to halve the risk of breast cancer in women with *BRCA1* or *BRCA2* mutations \(^{13-15}\). This suggests that endogenous hormones play an important role in the etiology of breast cancer among mutation carriers. However, the effect of exogenous hormones on the risk of breast cancer in *BRCA1/2* mutation carriers is controversial \(^{16-17}\).

Chapter 5 describes the association between use of oral contraceptives and the risk of breast cancer using the International *BRCA1/2* Carrier Cohort Study (IBCCS) comprising 1,593 *BRCA1/2* mutation carriers \(^{18}\). A significant association was found between use of oral contraceptives and the risk of breast cancer was found (HR=1.47, 95%CI=1.16-1.87). In contrast with the general population current use of oral contraceptives was not associated with an increased risk of breast cancer and there was no decrease in breast cancer risk and there was no decrease in breast cancer risk and there was no increase in breast cancer risk. There was no risk increase of prostate and pancreatic cancer. Reduced risks were found for several sites, including rectal (RR=0.22, 95%CI=0.10-0.45) and lung cancer (RR=0.83, 95%CI=0.69-1.00), melanoma (RR=0.47, 95%CI=0.21-0.89) and kidney cancer (RR=0.26, 95%CI=0.13-0.47). This study confirms increased risks of cervical cancer and endometrial and possibly stomach cancer in *BRCA1* mutation carriers. At an increased risk for colorectal cancer was only observed in women, elevated risks may be due to misreporting of ovarian cancer. In contrast to some previous studies, no increased risks were observed for prostate cancer at a young age or pancreatic cancer.
In Chapter 6 the IBCCS cohort was extended to 3,364 BRCA1/2 mutation carriers to study the association between hormonal replacement therapy (HRT) use and breast cancer risk among BRCA1/2 mutation carriers with a natural menopause, using the weighted cohort approach. To adjust for the potential survival bias a pseudo-incident cohort was selected comprising breast cancer cases diagnosed within the last five years before interview. Among 224 BRCA1/2 mutation carriers with a natural menopause, ever HRT use was not associated with risk of breast cancer (adjusted HR = 1.42; 95% CI = 0.68-2.96). In the general population, an increasing risk of breast cancer was observed when the start of HRT use was closer to the age of onset of menopause. The increased risk for HRT use within 5 years since natural menopause (adjusted HR=2.78; 95% CI=1.21-6.38) suggests that the timing of HRT use is important for BRCA1/2 mutation carriers as well. Thus, before deciding on HRT use after natural menopause, BRCA1/2 mutation carriers should be informed about the potential risks (and benefits) of HRT use. However, results from large prospective studies are needed to be able to give unequivocal advice to BRCA1/2 mutation carriers with regard to the use of oral contraceptives and HRT.

3. Methodological considerations

3.1. General methods and study population
The probability that a woman carrying a mutation will develop the disease by a given age is referred to as penetrance. Deriving the appropriate estimates of breast and ovarian cancer penetrance in BRCA1/2 mutation carriers is crucial in genetic counselling and clinical management of high-risk women. Based on this information, women should be able to make decisions on screening and risk reducing surgery. Therefore, an important objective of this thesis is to provide accurate estimates of breast and ovarian cancer penetrance in women with a BRCA1 and BRCA2 mutation. Numerous, mainly retrospective, studies have been conducted to estimate breast and ovarian cancer penetrance in BRCA1/2 mutation carriers with varying success. Generally, they can be divided into:

1) family-based studies using segregation analysis;
2) case-family studies using data from relatives of identified mutation-carrying breast cancer cases unselected for family history or kin-cohort studies;
3) population-based studies comprising case only, case-control or cohort studies.

Important to note is that all such studies used data reported by relatives to estimate cancer risks.

This thesis comprises a retrospective analysis of 758 BRCA1/2 families to estimate cancer risks associated with BRCA1 and BRCA2 mutations and an analysis of 3,364 mutation carriers to assess the role of hormonal factors as potential modifiers of cancer risks in BRCA1 and BRCA2 mutation carriers. The families were recruited at eight clinical genetic centres across the Netherlands during the period 1997-2006. Initial mutation screening was performed in families meeting one of the following criteria:

a) three or more first-degree relatives of breast and/or ovarian cancer in two successive generations;
b) two first-degree relatives diagnosed with breast cancer in which one had been diagnosed before the age of 45;
c) two affected first-degree relatives, where one case had been diagnosed with pre-menopausal breast cancer and the other with ovarian cancer;
d) breast cancer and ovarian cancer in a single individual with breast cancer diagnosed before the age of 60;
e) breast cancer before the age of 36;
f) families with a male individual diagnosed with breast cancer;
g) two first-degree relatives diagnosed with ovarian cancer.

To estimate risks in families and in a selected cohort of carriers two major types of biases should be taken into account i.e., ascertainment and testing bias. Ascertainment bias may occur as a result of selecting high-risk families with multiple cases of breast and ovarian cancer seen in clinical genetics centres. Testing bias occurs when affected and unaffected carriers are likely to be sampled with different probabilities at different ages because genetic testing is primarily targeted at affected individuals diagnosed at an early age. Therefore, the selection of families and mutation carriers in our cohort is non-random with respect to disease status and the carriers in our study did not represent a true cohort.

In this section these major types of biases and the methods used to account for these biases will be explained when using families and a cohort of mutation carriers to estimate cancer risks. The strengths and limitations of the maximum likelihood method to estimate the penetrance in BRCA1/2 families are discussed in Section 3.2. In Section 3.3 advantages and disadvantages of the kin-cohort approach to estimate cancer risks on cancer sites other than breast and ovary are described. In Section 3.4 the potential biases to estimate breast cancer risk associated with hormonal risk modifiers in BRCA1/2 mutation carriers are addressed.

3.2. Estimating penetrance in BRCA1/2 families using the likelihood method
In this thesis breast and ovarian cancer risks were estimated by maximum likelihood using modified segregation analysis implemented in the computer program MENDEL. The likelihood approach provides a method for calculating unbiased estimates, though it may not always be straightforward and can be difficult. MENDEL internally incorporates the Elston-Stewart algorithm to estimate the conditional likelihood of each pedigree, to solve a variety of problems including ascertainment correction. Ascertainment bias may become a problem when the estimated risks depend on the setting from which carriers and their relatives were sampled (i.e. external validity, families not representative for all BRCA1/2 families in the population). Another source of ascertainment bias may occur if ascertainment index cases is dependent on factors that are related to the disease (i.e. internal validity, cancer history of the index case or family history at the time of testing the index). For example, initial estimates of breast and ovarian cancer risks conferred by mutated BRCA1/2 genes may have been overestimated because the study population consisted of women from multiple-case families selected by the Breast Cancer Linkage Consortium (BCLC) to identify high-risk loci. Thus, a correct analysis of penetrance should take into account the process by which the families or carriers included in the analysis were sampled. Failure to do so can lead to upward biased estimates.

The maximum likelihood method used in the present study can be considered as a modified segregation analysis and is only worthwhile if the method of ascertainment is known and systematically and accurately adjusted for in the analysis. Ascertainment correction was
achieved by maximizing the conditional likelihood of each pedigree given all phenotypic information of the index family and the genotypic information of the first typed carrier in the family or index carrier. Thus, the strength of the maximum likelihood method to estimate the penetrance in BRCA1/2 families is that all genotype and phenotype information of the family is used, while potential ascertainment bias can be avoided. The MENDEL likelihood approach has been applied in family-based studies by the BCCL and others, and the meta-analysis conducted by Antoniou et al in 2003, comprising cases unselected for family history.

Strengths and limitations
The strengths of this penetrance study are the large sample size and the extensive genotyping performed within the families. This is an important issue as in most of the family-based and population-based studies, risk estimates were based on the incidence of cancer in relatives with unknown mutation status rather than in tested carriers.\textsuperscript{2,24,30}

To our knowledge this is also the largest study with a clinically-ascertained nationwide series of BRCA1/2 families. The dataset may represent all possible family cases seen in clinical genetic centers up to 2005 throughout the Netherlands. Novel aims compared to other studies included the investigation of risks in one population with a unique spectrum of mutations and mutation prevalence.

We estimated breast and ovarian cancer risks using MENDEL incorporating all available genotypic and phenotypic information in the families. An additional strength of the present work, as compared to others, is that the risk-reducing effect of oophorectomy on breast cancer was taken into account in the analysis by censoring on oophorectomy. Moreover, individuals were censored on breast cancer, ovarian cancer or another cancer, whichever occurred first, to avoid potential treatment effects on the outcome. But with 18% of the ovarian cancer cases diagnosed after breast cancer, this approach reduces the power to estimate the risk of ovarian cancer and may underestimate this risk.

In our study, we observed cumulative breast cancer risks up to age 70 of 45% (95%CI=36-52%) for BRCA1 mutations and 27% (95%CI=14-38%) for BRCA2 mutations and ovarian cancer risks of 31% (95%CI=17-43%) for BRCA1 mutations and 6% (95%CI=2-11%) for BRCA2 mutations. Our risk estimates are substantially lower than those estimated in the meta-analysis by Antoniou et al and other family-based studies\textsuperscript{2,3,24}, see Table 1. Several issues may explain the lower risk estimates. These include:

a. Unknown incidence rates before 1960
The first problem concerns the unknown cancer incidence rates in the older cohorts or those born before 1960. In the analysis penetrance is derived from the age-specific relative risks. These relative risks were calculated by comparing the observed cancer incidences in this cohort with the expected site-, sex-, and period-specific incidence rates. Given the stable mortality rates of especially breast cancer in the period 1950-1990, and the improving survival rates in that period, it is likely that the incidence rates before 1960 are significantly lower than those after 1960. In the analysis the incidence rates of 1960 were used for the total period preceding 1960. We may have overestimated these rates and underestimated the RR for the older cohort. We therefore performed a sensitivity analysis incorporating age- and period-specific incidence rates before 1960 extrapolated from the known estimated mortality data in that period. We used the lethality ratio as the ratio of mortality and incidence to calculate age-specific fatality rates. With these fatality rates the age- and period-specific incidence rates were imputed from the mortality rates in the periods before 1960. This method indeed resulted in lower incidence rates than the rates that were originally used in the model. However, when using these imputed incidence rates in the model the estimated cumulative risk of breast cancer risk was 46% at age 70 years, quite similar to the original estimated risk of 45%. Thus, apparently the very old birth cohorts did not contribute much to the overall risk estimates.

b. Over-adjustment by ascertainment correction
In a clinical genetic setting usually the youngest affected female is tested first and denoted as the index carrier. To correct for ascertainment, we adjusted for the phenotype of the family and the genotype and phenotype of the index carrier. This choice of conditioning was based on the assumption that subjects were ascertained because they had a family history of breast and ovarian cancer and/or their own cancer history. Since the age at diagnosis of the index case mutation carriers was significantly lower compared to the other typed carriers, excluding the index carrier from the analysis as we did through our ascertainment correction, may have resulted in an over-correction of early onset cases and partly explain the low estimates. Unless one identifies the exact ascertainment of each family, the ascertainment correction used in this thesis is the most conservative way of correction. We performed analyses with and without ascertainment correction and found that the ascertainment correction as expected decreased the risk estimates.

c. Missing data and misclassification of cancer cases
Family-based studies have to rely to some extent on self-reports from relatives of family history of the disease. Misclassification and inaccurate reporting might cause discrepancies among studies as a complete and accurate family history is crucial for unbiased estimates. Murof et al. examined among first-degree relatives the accuracy, sensitivity and specificity of self-reports and showed that the accuracy of information depends on the disease\textsuperscript{32,33}. Reports among first-degree relatives of affected individuals that can be relatively easy diagnosed such as breast cancer are reliable, but for cancers difficult to be recognized and diagnosed, such as ovarian cancer the self-reported family history appears to be less reliable.

Misclassification may concern several aspects: (1) phenotypic information such as misdiagnosis of the type of cancer, underreporting of cancers, over reporting of non-invasive lesions as invasive cancers, and mis-specified the age at diagnosis; (2) pedigree information such as maternal and paternal information, gender, number of relatives and dates of births, prophylactic surgeries and deaths of relatives; (3) genotypic information i.e., relatives that are incorrectly identified as carrier or non-carriers of the putative mutation that runs in the family. The present study was performed among clinically ascertained families. Accurate information on malignancies was obtained through pathology confirmation of the reported cancers of distant relatives was obtained through interviewing relatives closer related to these family members. Furthermore, in the present study design families were visited at home. During this visit, pedigrees were updated and validated, while relatives were interviewed about their cancer history, family history, hormonal/lifestyle exposures. Moreover, our family data set was linked with the
Netherlands Cancer Registry and Netherlands Pathology Database (PALGA) to increase the confirmation rate of cancers in our cohort.

Missing information on date of birth, age at diagnosis and age at death were imputed following a standard protocol and each pedigree structure was validated with the program PedCheck. Sensitivity analyses were performed to examine the influence of the imputation and the potential underreporting of cancers in distant relatives or older generations. This did not appear to be a problem, because analysis restricted to first-degree relatives of tested family members gave similar results.

d. Risks for non-carriers of BRCA1/2 mutations

The likelihood model is based on the assumption that cancer risks for non-carriers of BRCA1/2 mutations follow the population-specific incidence rates. This assumes that the increased cancer risks are explained by the two major genes without taking into account a potential polygenic factor. Because this factor can be present in both carriers and non-carrier, it is more likely that non-carriers have a higher cancer risk in BRCA1/2. To date, there is conflicting evidence as to cancer risks among women who test negative for the BRCA1/BRCA2 mutation segregating in the family. A possible increased risk of breast cancer has been reported for these women, whereas small prospective studies showed no increased risk. Thus, more data as to the risk among non-carriers are needed to examine the influence of a polygenic factor in the likelihood model.

e. Effects of birth cohort and family history

Several studies have indicated that the penetrance of BRCA1 and BRCA2 mutations have increased in recent generations, indicating that non-genetic factors influence cancer risks. Our results provide further evidence that, belonging to a more recent birth cohort and having a strong breast cancer family history are both associated with higher risks of breast and ovarian cancer in BRCA1/BRCA2 mutation carriers. This was not observed among BRCA2 mutation carriers which could be due to less power. These observations suggest that the inclusion of relatively more family members from older birth cohorts and families with a less strong family history, may explain the lower breast and ovarian cancer risk estimates found in this thesis compared to the estimated risks, for instance, in the meta-analysis reported by Antoniou et al. The differential effects of birth cohort on the risks of breast and ovarian cancer between BRCA1 and BRCA2 mutation carriers may suggest that BRCA1 expression may be more susceptible to reproductive or hormonal factors changing over time.

3.3. Risk of cancer at sites other than breast and ovary

In 517 BRCA1 and 139 BRCA2 families we studied whether cancers at sites other than breast and ovary are associated with the BRCA1/2 genotype. In the present study risks of cancers other than breast and ovary were estimated using a kin-cohort of 50% presumed carriers to minimize potential testing bias. In this kin-cohort the standardized incidence ratio was calculated as the ratio of observed cancers in this cohort to expected cancers derived from the age-, sex-, calendar period- and site-specific cancer population incidence rates. A disadvantage of this approach is that not all pedigree information is used, including the large number of typed carriers. The obvious alternative approach is the likelihood method. Unfortunately, it was not possible within the timeframe of this thesis to further explore this possibility. The most important limitation of both studies, described in Chapters 3 and 4, is that only 43% and 46% of all cancers at known sites for BRCA1 and BRCA2 were histologically confirmed. As was described before, reported cancer histories of relatives are mostly accurate for cancers such as breast cancer, but for other cancers the reported family history may be less reliable. For example, brain and liver cancer might be explained as distant metastases from breast and ovarian cancer, or reported colon cancer in a family member may be misclassified ovarian cancer. Another limitation of the present studies is that risk factor information was not available for all family members. Differences in risk factor exposure might contribute to some of our findings. For example, HPV infections may have caused the increased risk of cervical cancer among female carriers. Moreover, the decreased risk of lung cancer found in BRCA2 families might be due to smoking cessation as a result of a more healthy behavior in these families.

3.4. Hormonal modifiers of cancer risks in BRCA1/2 mutation carriers

The studies presented in Chapters 5 and 6 are retrospective analyses of 1,601 and 3,365 BRCA1/2 mutation carriers recruited during the period 1997-2005. To date, the designs of studies examining modifiers of BRCA1/2-related cancer risks include case-control and cohort studies among BRCA1/2 mutation carriers. These studies suffer from more difficulties than similar observational studies conducted in the general population. The problems are largely related to the reasons underlying identification of carriers of the disease-causing mutations. Testing and survival bias are discussed below. Another important limitation of our design is that we included mutation carriers only, implying that gene-environment interactions could not be properly studied. A valid study of interactions between genes and risk factors as potential effect modifiers should include both carriers and non-carriers. So far, only a few studies have been conducted that truly examined gene-environment interactions. All studies had very limited power, because the event rate of breast cancer and ovarian cancer in non-carriers is low whereas, the case-control studies relied on assumptions that could not be easily tested.

The variation in cancer risks suggests that gene expression is influenced by multiple factors. The effect of each separate factor is likely to be small which limits statistical power. Moreover, each risk factor has its own difficulties related to exposure assessment and potential confounding by indication. Therefore in the interpretation of the study results we carefully evaluated the methodological limitations.

Testing bias

Standard Cox regression to estimate the penetrance of breast and ovarian cancer in BRCA1 and BRCA2 families leads to biased estimates of the HR because the women in the studies described in this thesis were selected from high-risk families qualifying for genetic testing. For affected women in such families, the likelihood of ascertainment and testing is usually higher than for unaffected family members. This may result in an oversampling of affected women among the tested BRCA1/2 mutation carriers in our cohort. To correct for this potential testing bias, analyses were performed using the weighted regression approach described by Antoniou et al. Briefly, this involves assigning different weights to breast cancer and ovarian cancer cases and unaffected individuals, such that incidence rates for breast cancer in the study cohort are consistent with established incidence rates in BRCA1/2 mutation carriers according to the meta-analysis of Antoniou et al. The weighting typically resulted in a slight shift of the risks away from zero and wider
One assumption underlying the method of weighting is that the decision to opt for genetic testing is similarly associated with use of oral contraceptives (OC) for cases and unaffected women. We have verified this assumption in the Dutch cohort by comparing OC use between tested and untested members of BRCA1/2 families for women with or without breast cancer, separately (50 affected pairs and 123 unaffected pairs, matched on age at date of testing). Although OC users proved to be more likely to opt for genetic testing than non-users, this testing behavior was comparable for women with and without breast cancer (HR=2.4; 95% CI=0.9-6.8 and 2.1; 95% CI=0.1 to 4.0, respectively). Thus, it is not likely that testing bias has still an impact on the association between OC use and breast cancer risk in a weighted analysis.

Survival bias or prevalence-incidence bias
To increase power, in most observational studies on the effect of risk modifiers on breast cancer risk in BRCA mutation carriers, the study population includes mutation carriers diagnosed with breast cancer in the past and who are still alive at the starting date of the study (prevalent cases). This may result in biased risk estimates only if the exposure of interest is associated with breast cancer survival (survival bias). If we assume that the risk factor under investigation increases breast cancer mortality, cases which were exposed to the risk factor are underrepresented when including prevalent cases, because many exposed cases had already died before the study started. As a result the relative risk may be underestimated.

Breast cancer survival has improved over the years so that more than 70% of the breast cancer cases diagnosed five years ago are still alive. To adjust for potential survival bias we restricted the Cox-regression analysis to the last five years preceding the date of interview. In this cohort the mean interval between dates of diagnoses and interview was reduced from nine years in the entire cohort to two years in the subcohort of women contributing person-time. This cohort included the most recently diagnosed cases and was therefore denoted as the ‘pseudo-incident’ cohort. Weights were estimated for the entire cohort and the ‘pseudo-incident’ cohort separately and the ‘pseudo-incident’ cohort analyses were additionally adjusted for their age at start of follow-up. The ideal study design would be a prospective study which does not have the problem of survival bias. However, an alternative approach is a case-control study. This resulted in lower but less precise estimates and could be prone to selection bias as well, while a difference in risks could be explained by modifying risk factors. Discussing all published studies of breast and ovarian cancer risks in BRCA carriers is beyond the chapter, but some should be mentioned to put the results into perspective.

4. Results in perspective: what did others find?

4.1. Penetration
Published estimates of breast and ovarian cancer risks in BRCA1/2 mutation carriers vary substantially. This variability in risks can be explained by differences in study populations (including mutation frequencies, mutation spectrum, family histories), study designs, modifying factors and chance. Initial penetrance estimates of BRCA1 and BRCA2 mutations were based on selected very high-risk families to identify disease loci. Risk of breast cancer at age 70 years for women carrying BRCA1 or BRCA2 mutations appeared to be as high as 85%, whereas ovarian cancer risk was 64% for BRCA1 and 27% for BRCA2 (see Table 1). Since these studies were based on multiple-case families, the reported risks may overestimate the risks in carriers with a more modest family history which are nowadays mostly seen for genetic testing. Therefore, alternative approaches were sought to estimate penetrance from population studies comprising cases unselected for family history. Because of the low prevalence of the two genes, this was, however, only feasible in population with a common founder mutation such as the Ashkenazi Jews and Icelandic populations. This resulted in lower but less precise estimates and could be prone to selection bias as well, while a difference in risks could be explained by modifying risk factors. Discussing all published studies of breast and ovarian cancer risks in BRCA carriers is beyond this chapter, but some should be mentioned to put the results into perspective.

In 1997, Struwing et al. reported the first Ashkenazi Jewish population-based study comprising volunteers from the Washington DC area in the U.S. The estimated risks by age 70 were 56% for breast cancer and 16% for ovarian cancer and this was the first study to show that the risks were lower than the risks found in high-risk families. With a rather straightforward statistical method this kin-cohort approach was highly recommended by epidemiologists to evaluate the risk of susceptibility genes. However, risk estimates were based on the reported cancer incidence or family history of carrier and non-carrier relatives rather than on confirmed cancers, while carrier status of relatives was inferred by applying the rules of Mendelian inheritance instead of direct testing. Cancer risks in non-carriers...
were higher than the population risks, which may have resulted from selecting volunteers with a positive family history. Struewing et al. reported a decreased risk of ovarian cancer among BRCA1 mutation carriers which may partly due reporting bias (e.g. misclassified ovarian cancer for colon cancer). Moreover, the carrier status (being a carrier, non-carrier or not tested at all) may have been misreported. Relatives incorrectly reported as non-carriers and the relatively high incidence rates in non-carriers, may have led to lower relative risks estimates when comparing carriers with non-carriers. Furthermore, in a selected ethnic founder population and a relatively small area, unrecognized overlap of family members may easily occur, resulting in biased estimates.

In an alternative population-based approach among Ashkenazi Jews, Santagopan et al. tested ovarian cancer cases and their controls for BRCA1/2 mutations52. The estimated penetrances of ovarian cancer at age 70 years were 37% (95%CI=25-71%) for a BRCA1 mutation and 21% (95%CI=13-41%) for a BRCA2 mutation. These estimates were inferred from the calculated odds ratio’s of being a carrier, mutation frequencies and population incidence rates. However, the use of the odds ratio as a relative risk to estimate penetrance may be invalid due to the low numbers of identified carriers. Other concerns involved the selection of hospital controls that may represent a more susceptible group. Moreover, in this study only living prevalent cases were included and mutations tested. Thus, potential survival bias may have influenced the study outcome. Alternatively, Hopper et al. used a population-based case family design to estimate cancer risks in relatives55. In this design cases and their relatives were recruited, as well as controls and their relatives, and data were analyzed using the likelihood method. Penetrance of BRCA1/2 carriers to age 70 was 40%, about half that estimated from BCLC families. This approach can be a powerful method for family-based and population-based studies.

Additional retrospective study designs were based on tested mutation carriers only. King et al. and de la Chapelle et al. Kaplan Meier survival analysis to relatives of mutation carrying breast cancer cases unselected for family history56. The cumulative risks of breast cancer by age 70 were estimated to be 69% for BRCA1 and 74% for BRCA2 mutation carriers (Table 1). However, there was a clear testing bias in these estimates, because only tested carriers were included in the study (see Chapter 3.4). As was mentioned before, testing bias may occur when genotyping is not random. In this setting, testing may depend on the disease status of the relatives, as living affected women are more inclined to go for testing. This may have caused a higher number of affected carriers than expected and could explain the high estimates found in this study. Finally, the naive practice of simply excluding the index case in a design using tested family members does not guarantee unbiased estimates, particularly in retrospective studies. Ascertainment-adjusted likelihood approach, as was used in thesis, can overcome the above mentioned problems and provides unbiased estimates. This method is, therefore, the best option to infer valid estimates from carrier and their relatives in a retrospective study design.

4.2. Cancers at sites other than breast and ovary

So far, risks of cancers at sites other than breast and ovary have been estimated in family-based studies and in population-based studies, mainly among Ashkenazi Jewish founder population, by comparing the BRCA1/2 founder mutation rates in cancer cases and their controls50,52,53. We could confirm the excess risks of endometrial and cervical cancer in BRCA1 mutation carriers (RRBRCA1=4.41 versus RRBRCA2=3.82 for cervical cancer and RRBRCA1=2.77 versus RRBRCA2=2.65 for endometrial cancer). In contrast to the family-based studies conducted by the BCLC, we observed no increased risks for prostate cancer at a young age (RRBRCA1=0.75; 95%CI=0.33-1.49 versus RRBRCA2=1.82; 95%CI=1.03-3.29) and pancreatic cancer (RRBRCA1=1.42; 95%CI=0.83-2.27 versus RRBRCA2=2.26; 95%CI=1.26-4.06) for BRCA1 mutation carriers54. Among BRCA2 mutation carriers, we could confirm the increased risks of prostate and pancreatic cancer. The present study confirms the increased risk of colon cancer in BRCA1 mutation carriers (RR=2.51; 95%CI=3.02-3.07) and decreased risk of rectal cancer as was reported in the two BCLC studies55,56. However, the markedly low number of rectal cancers suggests that some rectal cancers have been incorrectly reported as colon cancers. Moreover, the statistically significantly increased risk of colorectal cancer found only in women suggests that part of the increased risk of colon cancer in BRCA1 mutation carriers may be due misdiagnosis of ovarian cancer as colon cancers. Thus, it is not likely that colon cancer is part of the BRCA1 phenotype, although further studies are needed with a higher confirmation rate of colon cancers. Our results may indicate that BRCA1 mutations are associated with gynaecological cancers, whereas prostate and pancreatic cancers appear to be more part of the BRCA2 phenotype. However, more studies are needed to examine the risk of prostate cancer among BRCA1 mutation carriers at a young age.

4.3. Hormonal modifiers

The marked reduction of breast cancer risk in BRCA1/2 mutation carriers following a prophylactic oophorectomy suggests that hormonal exposures play an important role in the etiology of BRCA1/2 related breast cancer14,57. In this thesis we examined whether oral contraceptive use and hormonal replacement therapy (HRT) modify breast cancer risk in BRCA1/2 mutation carriers. Only a few studies have addressed these issues with inconsistent results. In this thesis a retrospective cohort design was used as part of the International BRCA1/2 mutation Carriers Cohort Study.

In the general population, current use of oral contraceptives is associated with a modest but increased relative risk of breast cancer risk of 1.24 and this risk gradually disappears after cessation58. Among BRCA1/2 mutation carriers several studies have examined the effect of oral contraceptive use on breast cancer risk16,56-61. In the present study ever use of oral contraceptives was associated with an increased risk of breast cancer (HR=1.47; 95%CI =1.16-1.87). Current use was not associated with the risk of breast cancer observed, but an increasing risk with longer durations of use before first full-term pregnancy was seen among both BRCA1 and BRCA2 carriers. These associations seemed to be stronger among BRCA2 carriers, which was in accordance with the findings of Haile et al.61 Among 981 case-control pairs of BRCA1 carriers, Narod et al. found a modestly increased risk for ever use (Odds Ratio (OR)=1.20; 95%CI=1.02-1.40) and an increased risk for duration of use only in BRCA1 carriers (OR=1.33; 95%CI=1.11-1.60)16. In contrast, Haile et al. found no association for ever use of OC, but stronger duration effects for BRCA2 mutation carriers who used oral contraceptives for five years or more (OR=2.66; 95%CI=1.08-6.43). Differences in designs to avoid potential testing bias could explain the differences in outcome. All studies were based on retrospective information from women who opted for genetic testing. In the present study the weighted regression approach was applied to adjust for the overrepresentation of cases among women who opted for a test. In the study of Narod et al. no attempt was made to avoid testing bias. Moreover, in the case-control matching every case in the study, cases
Estrogens and progestogens, breast cancer and the role of BRCA1

Estrogens and progestogens are essential for the normal functioning of a woman’s reproductive system and for normal breast development. They are also crucial to maintain the bone density and a healthy cardiovascular system. Lifetime exposure to estrogens and progestogens is thought to increase a woman’s risk for breast cancer.56,69 Although, progestosterone action in breast cancer is grossly understudied, it remains controversial. Therefore, it has been proposed that most of the established risk factors influence breast cancer risk through a hormonal-related pathway mostly regulated by circulating gonadal hormones and postmenopausal estrogen production from adipose tissues.29

Estrogens have been implicated in the etiology of breast cancer, because of their role in stimulating breast cell proliferation and differentiation, their effects on other hormones that stimulate proliferation and their role in the growth of estrogen-responsive tumors. Related to the latter effect, clinical trials have shown that the anti-estrogen agonist tamoxifen effectively reduces the risk of primary breast cancer and recurrence of breast cancer.71 Furthermore, epidemiological studies in the general population suggest that the longer the exposure to endogenous estrogens, the higher the risk of breast cancer.22,73 This observation was supported by the finding that the removal of the ovaries at a young age, markedly reduced breast cancer risk.24,76

It has been suggested that estrogens affect breast cancer risk according to a dual mechanism in which they have a stimulating and a reverse effect on breast cancer development.77 In the proposed model high circulating estrogen levels originating from the environment, adipose tissue and ovaries stimulate breast cells to proliferate and differentiate. In this process estrogens activate tumor suppressor genes in the normal breast of young women, which then reduce and eliminate possible genetic alterations. Thus, reduced breast cancer risk can be achieved by estrogen-induced activation of tumor suppressor genes. However, when the breasts contain transformed cells and mutated or inactivated tumor suppressor genes, the genes are not able to correct adequately all genomic errors. In this way estrogens further promote the growth of existing transformed cells, leading to the development of breast cancer.

Strong candidate estrogen-regulated tumor suppressor genes are BRCA1 and p53, which was confirmed by findings of Marquis et al. and others.65,79 They found that BRCA1 expression is increased during puberty and pregnancy and is found in rapidly proliferating cells undergoing differentiation in response to high levels of estrogens. Thus, high-estrogen levels induce normal BRCA1 expression to ensure genomic stability as a response to an estrogen-induced breast cell proliferation and differentiation during puberty and pregnancy.

The observation that up to 90% of the breast cancers in BRCA1 mutation carriers are estrogen receptor negative has led initially to the suggestion that hormones are not relevant for the BRCA1-related carcinogenesis.60 However, the risk reduction after prophylactic oophorectomy suggests that estrogens are important in the early development of BRCA1-associated breast cancer.77 There is emerging evidence that in addition to the established role in DNA repair, BRCA1 regulates the ER-alpha and the androgen (AR) receptor expression and activity.81 In vitro studies have shown reduced BRCA1 expression, during the transition from carcinoma in situ to invasive breast cancer, because of loss of the wild type allele of the BRCA1 gene.62,83 Since BRCA1 regulates the ER-receptor expression and activity, reduced BRCA1 expression may result in loss of ER-expression and finally to the development of tumors that are ER-negative. This had led to the hypothesis that in BRCA1 mutation carriers breast cancer follows a distinct pathway in which estrogen stimulation is required early, followed by loss of estrogen receptors later on.

The potentially different effects of estrogens on breast cancer development in BRCA1 and BRCA2 mutation carriers may also be deduced from the change of the age-specific risk throughout life. This thesis and other publications showed that the age-specific cumulative risks of breast cancer differ between BRCA1 and BRCA2 mutation carriers.4 We confirmed that the age-specific relative risks of breast cancer risk among BRCA1 mutation carriers decreases with age from >30-fold in women 40 years old to 4-fold in women >60 years old (P for trend<0.001). In contrast, compared to the general population, the age-specific relative risk for women with a BRCA2 mutation was on average 5-fold and was constant over all age categories. This risk was lower at younger ages compared to BRCA1 mutation carriers. This finding may reflect differences in the age-specific susceptibility of the breast to
hormones among mutation carriers.

The combined effects of estrogen and progesterone-induced cell proliferation and loss of the BRCA1-DNA repair mechanism by mutated BRCA1 in inherited breast cancer or down-regulated BRCA1 by methylation\(^6\) in sporadic breast cancer may increase the likelihood that an accumulation of mutations will occur that lead to breast carcinogenesis. This interaction between estrogens and BRCA1 probably explains, at least in part, why BRCA1 mutation carriers exhibit an increased risk of breast cancer and other estrogen-regulated sites including ovaries, cervix, uterus and possibly the colon, but not in non-estrogen-regulated tissues.

5. Clinical implications

BRCA1 and BRCA2 are high-risk breast and ovarian cancer genes showing incomplete penetrance. This indicates that not all mutation carriers will develop the disease and that other factors, either genetic or non-genetic, can modify the risks. Improved knowledge of the heterogeneity in cancer risks will contribute to more accurate counseling of mutation carriers about their risks and potential means of reducing their risks.

In this thesis, the average cumulative risks of breast cancer and ovarian cancer by the age of 70 years were 45% and 31%, respectively, for BRCA1 mutation carriers and 28% and 7%, respectively, for BRCA2 mutation carriers. Although, the average breast and ovarian cancer penetrances may not be as high as expected from other studies (see Table 1), they are substantial, both in relative and absolute terms. In this thesis we found further evidence that the risks vary by birth cohort and family history among BRCA1 mutation carriers. BRCA1 mutation carriers from more recent birth cohorts and BRCA1 carriers with a stronger family history showed higher breast and ovarian cancer risks. But for BRCA2 mutation carriers, this variation in risk was less clear and needs to be further investigated with larger power. Our study also confirms that the penetrance estimates for breast and ovarian cancer are higher for BRCA1 mutation carriers than for BRCA2 mutation carriers. Thus, when counseling BRCA1 mutation carriers about their risks, the specific gene and the effects of birth cohort and family history need to be taken into account. For example, Table 2a shows the age-specific risks of breast cancer based on the overall risk estimates. Table 2b also shows the excess risks when taking into account the birth cohort effect. In the near future, we will further develop these models to include other genetic factors, mutation position and hormonal/lifestyle factors to provide a tool for personalised counselling of mutation carriers.

The maximum likelihood approach used in this thesis provides unbiased risk estimates for mutation carriers, because it takes into account ascertainment and family history. For the purpose of genetic counselling of high-risk individuals, the BOADICEA web-based program developed in Cambridge, can provide valid risk estimates\(^6\). Our estimates proved to be close or within the range of the BOADICEA program, which used the maximum likelihood method, and includes the family history as well. However, to provide accurate risk estimates for female carriers in the Netherlands, the model should be validated with data of an independent sample of Dutch BRCA1/2 mutation carriers using population-specific breast and ovarian cancer incidence and mortality rates. Moreover, the model should be extended to include hormonal/lifestyle factors.

We could confirm the excess risks of cervical, endometrial and possibly stomach cancer in BRCA1 mutation carriers. In contrast to previous studies, no increased risks were observed for prostate and pancreatic cancer. Among BRCA2 mutation carriers we could confirm the increased risks of prostate and pancreatic cancer. When these malignancies occur before age 65 in high-risk families, the counsellor needs to be aware that these tumors might be associated with a germline mutation in BRCA1 or BRCA2. BRCA1 and BRCA2 mutation carriers can be advised to adhere to the available population-screening recommendations. For example, BRCA1 mutation carriers can follow the screening guidelines for cervical cancer. However, for pancreatic cancer there is no proven effective screening method available so far. A nationwide study is being conducted to evaluate potential screening methods for pancreatic cancer, which may be important for BRCA2, but probably not for BRCA1 mutation carriers. The international targeted prostate screening study of men at increased prostate cancer risk (IMPACT study) examines new screening methods in BRCA1/2 mutation carriers, and may provide effective screening guidelines for men carrying BRCA2 mutations. However, based on our results, targeted prostate cancer screening does not appear to be relevant for BRCA1 mutation carriers.

The early BCRC study and several other studies suggested an increased risk of colon cancer among BRCA1 mutation carriers. In this thesis we could not confirm this association. The higher risk of colon cancer among BRCA1 mutation carriers is possibly due to mis-classification of this cancer, indicating that it is too soon to advise BRCA1 mutation carriers to adhere to specific screening methods for colon cancer.

It has been shown that estrogens and progestogens play an important role in the development of breast cancer. Depending on the type of hormone, prolonged or increased exposure may increase the risk of developing breast cancer, while reduced exposure may be protective. Although oral contraceptives have been shown to be protective against ovarian cancer in BRCA1/2 mutation carriers, this thesis and other publications demonstrate that their use may increase the risk of breast cancer among BRCA1/2 mutation carriers. Although prospective studies are needed to confirm these findings, certain types of oral contraceptives might not be suitable for BRCA1/2 mutation carriers. First, the copper Intra Uterine Device (IUD) might be a good option for these women, as it is clearly not related with the risk of breast cancer. An IUD does not protect women against ovarian cancer, but in a country with such a high uptake of BPSO as in the Netherlands that might not be an important consideration. The alternative option might be to use the new pill types with low-dose estradiol and a derivative of natural progesterone first (during adolescence when needed then), and then switch to a copper IUD later.

We found that postmenopausal use of HRT increased the risk of breast cancer among normally menopausal women who used HRT shortly after menopause. Our results indicate that HRT use after BPSO may attenuate the protective effect of BPSO, but power was low. Thus, high-risk women should be made aware of these potential effects when using or starting to use HRT, especially with long durations. Obviously, studies with more power and including women with longer duration of use are needed to understand the association between HRT and breast cancer risk in BRCA1/2 mutation carriers. As so far, the findings in BRCA1/2 mutation carriers are consistent with the findings in the general population. It might be assumed that similar effects apply to the carriers until proven otherwise.
These effects include (1) a slightly increased risk of breast cancer during use, (2) higher risk for continuously combined use of estrogen and progestogen compared to estrogen alone, and (3) increasing risk with longer duration of use.

6. Future research

For future studies on risk assessment the HE BON dataset could be updated with newly diagnosed cancers, prophylactic surgeries, genotype information, breast density and new families. There is a strong need for prospective studies to estimate breast and ovarian cancer risks and to examine the role of exogenous hormones on BRCA1/2 associated breast cancer risk in women who were unaffected at enrolment. This would eliminate many of the biases discussed in this thesis. Since only unaffected carriers will be included in a prospective study design most of the potential testing and survival bias can be avoided. The majority of carriers in such a study will come from high-risk families. As family history can influence cancer risks in mutation carriers, a prospective study design should therefore also include family history to estimate cancer risks conditional on ascertainment or family history. The likelihood approach or the BOADICEA model has been shown to be a useful and valid method to estimate these risks taking into account polygenic component based on family history. As was mentioned before, the model should be validated with data of an independent sample of Dutch BRCA1/2 mutation carriers and population-specific breast and ovarian cancer incidence and mortality rates. Additionally, the model should be extended with hormonal/lifestyle factors. Future studies should also include family members who are non-carriers of the mutation segregating in the family to examine gene-environment interactions (i.e., the interaction between BRCA1/2 genes and hormonal/lifestyle factors).

It is expected that in the CIMBA study polygenic susceptibility among BRCA1/2 mutation carriers will be further explored. In the future a dataset combining information on BRCA1/2 mutations, common variants, family history, hormonal/lifestyle factors and breast density is needed to establish a risk prediction model for BRCA1/2 mutation carriers. In this international study it is possible to examine the combined effects of non-genetic or hormonal/lifestyle risk factors on breast and ovarian cancer risks in BRCA1/2 mutation carriers. The international setting is needed to achieve sufficient events among non-carriers and also to have sufficient prospective events among mutation carriers.

To date, there are more than 2,800 typed BRCA1/2 families in the Netherlands and half of them are families that will harbour a known founder mutation (F. Hogeervorst, personal communication). Once an extended dataset including all families has been completed, it will be possible to examine potential genotype-phenotype correlations or mutation-specific risks with more statistical power and additional statistical modelling.

Finally, it has been shown that the genetic susceptibility of the majority of the families tested for the known high-risk genes remains unexplained. In a new international collaborative effort the polygenic factor can be further explored including BRCA1/2 and non-BRCA1/2 families. In a prospective study design ascertainment should be well-documented and follow-up well-organized including the collection of blood, tissue samples and information on hormonal/lifestyle risk factors. A powerful analytic tool will be required to model the combined effects and possible interactions between genetic and non-genetic factors. Through the international collaborations, knowledge on cancer risks in both BRCA1/2 and non-BRCA1/2 families will continuously be improved. This will be reflected in a risk prediction model that will be simultaneously updated. Given the difficult decisions women in high-risk families have to make on screening and prophylactic surgery, this information is urgently needed and will be directly applied in the clinical genetic practice.
Table 1. Reported cumulative risks of breast or ovarian cancer at age 70 for BRCA1 and BRCA2 mutation carriers

<table>
<thead>
<tr>
<th>Study*</th>
<th>Source</th>
<th>Study Population</th>
<th>N fam</th>
<th>Breast cancer CR (95% CI)</th>
<th>Ovarian cancer CR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Easton (1995)²</td>
<td>Multiple-case families</td>
<td>Family</td>
<td>33</td>
<td>85% (-)</td>
<td>63% (-)</td>
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<tr>
<td>2. Ford et al (1998)³</td>
<td>Multiple-case families</td>
<td>Family</td>
<td>280</td>
<td>66% (44-78)</td>
<td>39% (18-54)</td>
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<tr>
<td>3. Antoniou (2003)⁴</td>
<td>Population/hospital-based</td>
<td>Carriers only</td>
<td>67</td>
<td>69% (-)</td>
<td>46% (-)</td>
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<td>4. King (2003)⁵</td>
<td>Hospital-based cases</td>
<td>Family</td>
<td>28</td>
<td>48% (22-82)</td>
<td>39% (30-50)</td>
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<tr>
<td>5. Scott (2003)⁶</td>
<td>Multiple-case families</td>
<td>Family</td>
<td>283</td>
<td>46% (39-54)</td>
<td>60% (64-71)</td>
</tr>
<tr>
<td>6. Chen (2006)⁷</td>
<td>Clinic-based families</td>
<td>Carriers/FDR</td>
<td>223</td>
<td>68% (65-71)</td>
<td>22% (0-40)</td>
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<tr>
<td>7. Evans (2008)⁸</td>
<td>Clinic-based families</td>
<td>Family</td>
<td>155</td>
<td>52% (36-69)</td>
<td>59% (54-64)</td>
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<tr>
<td>9. Kolk (2010)¹⁰</td>
<td>Clinic-based families</td>
<td>Family</td>
<td>582</td>
<td>45% (35-52)</td>
<td>-</td>
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<tr>
<td>10. Present study</td>
<td>Clinic-based families</td>
<td>Family</td>
<td>76</td>
<td>-</td>
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* Depicted are larger studies with families or index cases from different populations and/or multiple centres.

Table 2a. Overall cumulative breast cancer risk in BRCA1 mutation carriers born after 1940

<table>
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<th>Age</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
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Table 2b. Overall cumulative breast cancer risk in BRCA2 mutation carriers born after 1940

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<th>Age</th>
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