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SUMMARY AND GENERAL DISCUSSION

AIM OF THE THESIS

The studies described in this thesis "Modifiers of breast cancer risk in BRCA1/2 mutation carriers" have provided information on the association of breast cancer risk with diagnostic radiation, physical activity, and body weight in BRCA1/2 mutation carriers. Furthermore, two methodological studies were conducted to provide information on potential non-differential and/or differential misclassification in the diagnostic radiation study.
After a short recapitulation of the main findings of the different studies, this chapter aims to identify methodological strengths and weaknesses and describe various aspects of the conducted studies (e.g. biological mechanisms and barriers encountered during data collection). Furthermore, conclusions are drawn and implications for clinical practice and recommendations are given.

MAIN FINDINGS

Studies have shown that penetrance, age at onset, and phenotypic expression vary between and within BRCA1 and BRCA2 families, suggesting that the development of breast cancer is also influenced by lifestyle and hormonal factors and/or genes other than BRCA1/2. Several studies have indicated that the penetrance of BRCA1 and BRCA2 mutations has increased in recent generations, which supports the concept that non-genetic risk factors, of which the prevalence has increased, also affect the risk[1-3].
The first study described in this thesis was a European collaborative retrospective cohort study (GENE-RAD-RISK) among 1,993 female BRCA1/2 mutation carriers from France, the United Kingdom (UK), and the Netherlands. The other four studies were all conducted among 1,120 female BRCA1/2 mutation carriers participating in the HEBON study (HEreditary Breast and Ovarian cancer study the Netherlands; see paragraph on study design below).
Chapter 2 reports on the association between self-reported exposure to diagnostic radiation and BRCA-associated breast cancer risk (N=1,993). Exposure to ionizing radiation is a concern in BRCA1/2 mutation carriers, as these women are frequently screened by mammography from a relatively young age onwards, while at the same time they might be more sensitive to ionizing radiation due to impaired DNA repair mechanisms. In BRCA1/2 mutation carriers, exposure to diagnostic radiation before age 30 was associated with an increased breast cancer risk at dose levels considerably lower than at which increases have been found in other radiation-exposed cohorts. A history of any diagnostic radiation before age 30 increased the risk of breast cancer 1.7-fold (95% Confidence Interval (CI)=1.20-2.48) and a dose-response association emerged: the risks for a cumulative dose of <0.002 Gy, ≥0.002-0.0066 Gy, ≥0.0066-0.0174 Gy, and ≥0.0174 Gy were 1.45 (0.92-2.27), 1.70 (1.03-2.79), 1.74 (0.89-3.39), and 3.29 (1.83-5.93), respectively. Because of the nature of our study population there was a special interest in the association between mammogram exposure and breast cancer risk. When compared to no exposure, a history of mammograms before age 30 increased the risk of

breast cancer 1.5-fold (HR=1.54, 95%CI=1.03-2.28) and a statistically significant dose-response association of increasing risk with greater number of mammograms emerged (P_{trend}=0.040). By sensitivity analysis, excluding carriers with exposure to mammograms, it was confirmed that our findings were probably not due to confounding by indication of family history.
An important limitation of the GENE-RAD-RISK study is the self-reported diagnostic radiation history. We relied on self-reports rather than medical record review because of the difficulties in accessing medical records with regard to the various diagnostic procedures, which occurred in many different contexts, especially when exposures occurred during childhood or in the distant past. In retrospective studies inaccuracy may lead to non-differential and/or differential misclassification, both of which could affect risk estimates. Non-differential misclassification, mostly resulting in bias towards unity, is inevitable in any study using subjects' information only. Inaccuracies that are systematically different between affected and unaffected individuals (i.e. differential misclassification or recall bias), can either mask true associations or create spurious ones. To investigate this potential source of bias, two methodological studies, described in chapters 3 and 4, were conducted in the Dutch part of the GENE-RAD-RISK study. Test-retest reliability of self-reported exposure to conventional radiographs (hereafter named X-rays), fluoroscopies and mammograms and validity of self-reported lifetime mammography history was assessed for the entire study population and by case-status. In affected BRCA1/2 mutation carriers lifetime exposure to diagnostic radiation was for a large part due to their breast cancer diagnosis. Such exposures are not taken into account in an epidemiological study such as the GENE-RAD-RISK study. Therefore, test-retest reliability and validity of *prediagnostic* mammograms was also addressed.
The test-retest reliability study (chapter 3), conducted among 401 BRCA1/2 mutation carriers who completed a baseline (1998-2003) and a follow-up questionnaire (2006-2007), showed that inconsistent self-report of exposure to diagnostic radiation by carriers was mainly non-differential by case-status. Overall proportion agreement on reporting ever/never exposure was good (>75%), while the corresponding kappa coefficients were between 0.40 and 0.75, indicating at least moderate reliability beyond chance. Reliability of number of exposures was also good (>75%). Proportion agreement on reporting age at first mammogram was low (40%) for exact consistency and moderate (60%) for consistency within 1 year. Reliability of age at first mammogram was higher for cases than for unaffected carriers (P<0.001) but this difference disappeared when analyzing prediagnostic mammograms only (P=0.60). In general, the direction of disagreement on all items was equally distributed. More consistent reporting was mainly determined by a younger age at questionnaire completion.
The validation study (chapter 4) was conducted among 218 BRCA1/2 mutation carriers and showed that the accuracy of self-reported lifetime mammography history widely varied, depending on the measure under investigation. However, the extent of the observed misclassification was small and mostly non-differential. Accuracy of reporting of ever/never exposure to mammograms was excellent (i.e. proportion agreement ≥90%) for all time frames (lifetime, before age 30, and at ages 30-39). Accuracy of

age at first mammogram was poor to moderate for exact agreement and improved to almost excellent (proportion agreement 70% and kappa 0.69) for agreement within 1 year, indicating that differences were small. Though cases more often tended to underestimate their exact age at first mammogram, while unaffected carriers tended to an overestimation, the difference in the direction of inaccuracy was not statistically significant (P=0.237). Carriers tended to underreport the time since last mammogram ("telescoping") and overreport the number of mammograms.

Because physical activity and body weight are two of the few more modifiable risk factors for breast cancer, they may provide a target to add to breast cancer prevention in a high risk population like BRCA1/2 mutation carriers. In chapter 5 (N=725 BRCA1/2 mutation carriers), we observed a statistically non-significantly decreased risk of breast cancer for ever engaging in sports activity (HR=0.84, 95%CI=0.57-1.24). Among women who had participated in sports, a medium versus low level of MET hours/week (i.e., between 11.0 and 22.7 mean MET hours/week averaged over a lifetime) reduced the risk of breast cancer (HR=0.59, 95%CI=0.36-0.95); no dose-response association emerged. For mean hours/week of sports activity, a statistically non-significant trend of decreasing risk of breast cancer with increasing mean hours/week was observed ($HR_{low\ versus\ never}$=0.93, 95%CI=0.60-1.43; $HR_{medium\ versus\ never}$=0.81, 95%CI=0.51-1.29; $HR_{high\ versus\ never}$=0.78, 95%CI=0.48-1.29; P_{trend}=0.272). For duration (i.e. number of years) of sports activity no statistically significant associations was observed. Among women active in sports before age 30, mean MET hours/week showed the strongest inverse association of all activity measures ($HR_{medium\ versus\ low}$=0.60, 95%CI=0.38-0.96; $HR_{high\ versus\ low}$=0.58, 95%CI=0.35-0.94; P_{trend}=0.053). Engaging in sports activity after age 30 was also inversely associated with breast cancer risk (HR=0.63, 95%CI=0.44-0.91). Our results indicate that BRCA1/2 mutation carriers may reduce the risk of breast cancer by participating in sports activity, preferably from early ages onwards.

The same study population was used to assess the association between body weight and breast cancer risk, which is described in chapter 6. Menopausal status seemed to modify the association between body weight and breast cancer risk among BRCA1/2 carriers. We observed no clear association between body weight and premenopausal breast cancer risk (N=609 BRCA1/2 mutation carriers). Among postmenopausal women (N=299 BRCA1/2 mutation carriers), a current BMI of ≥25.0 kg/m^2, an adult weight gain of 5 kg or more, and a relative adult weight gain of 20% or more were all statistically non-significantly associated with a 50-60% increased risk of breast cancer (HR=1.46 (0.86-2.51), HR=1.56 (0.85-2.87), and HR=1.60 (0.97-2.63), respectively), as compared to having a healthy or stable weight. Carriers may reduce their risk of postmenopausal breast cancer by maintaining a healthy body weight throughout life.

METHODOLOGICAL CONSIDERATIONS

STUDY DESIGN

The HEBON study is an ongoing nationwide cohort study among members of all families with a BRCA1/2 mutation in the Netherlands. The HEBON study was initiated in 1998 by the Departments of Epidemiology and Molecular Pathology of the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital. The main aim was to investigate cancer risk in familial and hereditary breast and/or ovarian cancer families. In addition, interaction between hormonal/lifestyle risk factors and cancer genes in the development of various cancers were to be examined. In 2006 a follow-up study was initiated. This study included a follow-up of BRCA1/2 families which had already participated from 1999 onwards and an expansion of the entire cohort (ascertainment of new families) (see Figure 4 in chapter 1). In 2009, a nationwide initiative to create a Research Facility for HEBON research was funded and started. The Facility will be based on data from the first national HEBON study and will consist of a central database with a limited set of clinical and genetic data, as well as coded identifiers that will enable linkage with related databases on genes, hormonal/lifestyle risk factors, treatment data, and with disease registries. The HEBON study is funded by the Dutch Cancer Society and from 2009 onwards by the Dutch Organization for Scientific Research.

The studies described in this thesis are among proven BRCA1/2 mutation carriers only. In order to assess true gene-risk factor interactions, a comparison between proven carriers and proven non-carriers is required. Based on the studies described in this thesis we could therefore only assess whether the risk factors under study affected breast cancer risk in carriers and whether these associations appeared to be of similar magnitude as observed in the general population.

A problem in most studies examining the influence of risk factors on breast cancer risk in BRCA1/2 mutation carriers is that they were still based on a retrospective design. The ideal study design would be a prospective study. However, with regard to the HEBON study, which was set up as a retrospective cohort study with a prospective follow-up planned in the future, it would have taken many years to obtain a large enough group to conduct a prospective study for two reasons. First, the BRCA1/2 DNA test has been available as of 1995; thus, unaffected carriers have not been followed very long. Second, the uptake of prophylactic surgery in the Netherlands is quite high (see chapter 1) resulting in relatively few incident breast cancer cases. In a traditional retrospective cohort study, a cohort of individuals is indentified based on their characteristics in the past and followed up for their subsequent disease experience up to the present[4].

A difference between a traditional retrospective cohort study and our retrospective cohort studies within HEBON is that we defined the cohort at the time of the DNA test, but the follow-up may start earlier (i.e. at birth or 5-10 years before questionnaire completion). Because many women tested after 1994 had been diagnosed with breast cancer in the past, prevalent cases are included, resulting in potential survival and/or recall bias. Furthermore, potential selection bias based on the DNA test (or: testing bias) may arise; women were selected from high-risk families qualifying for genetic testing

and the disease status (i.e. case-status) may therefore have increased the likelihood of ascertainment leading to an oversampling of affected women.
The HEBON study is subject to a combination of several types of biases, which, together with other methodological considerations, are discussed in the next few paragraphs.

SELECTION BIAS

Ascertainment bias based on selection of high-risk families

BRCA1/2 penetrance estimates from family-based studies[5-7] were higher (68-85% for BRCA1 and 75-84% for BRCA2) than estimates from population-based studies (45-65% for BRCA1 and 40-45% for BRCA2)[1,3,8,9]. The BRCA1/2 families in the HEBON study are identified through Clinical Genetic Centres. These families are self-referred based on their family history or personal history of breast and/or ovarian cancer. Therefore, the families included in the HEBON study are not necessarily a random selection of the background population of BRCA1/2 families in the Netherlands. It is possible that, after genetic testing became available in 1995, BRCA1/2 families with the strongest family history of breast and/or ovarian cancer were the first to be identified. Since 1995 the proportion of BRCA1/2 families identified in newly tested families declined from 30% to 20%, illustrating that the required level of family history to qualify for genetic testing, has declined (dr. Hogervorst, department of Pathology, Netherlands Cancer institute, Amsterdam, the Netherlands, *personal communication*). Thus, nowadays more lower-risk families are being tested. Even so, small families and/or families with the majority of offspring being male and/or lower-risk families (if different genotype-phenotype) are less likely to present themselves. As a result, ascertainment based on certain degree of family history may have occurred. This selection may bias the results if family history is related with the exposures that have been studied. For ionizing radiation, physical activity, and body weight this may not be very likely.

Testing bias

The cohort was defined at the time of the DNA test, but the follow-up in the analyses starts at birth. Standard Cox regression 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 and the case-status may therefore have increased the likelihood of ascertainment leading to an oversampling of affected women. Apart from the family selection, some of the affected women may be actively approached by the clinical geneticist after an unaffected family member sought genetic counseling as a first member in the family; because the first test in a family is very laborsome, it is important to select the family member with the highest change of being a carrier, i.e. a (young) breast cancer case. As a result, the risk factor profile of cases is overrepresented among the unaffected person-years in the cohort. To correct for this potential bias (hereafter referred to as testing bias), analyses were performed using the weighted regression approach described by Antoniou et al.[10] in which cases and unaffected individuals are differentially weighted so breast cancer incidence rates in the study cohort were

consistent with true age- and birth cohort-specific breast cancer risk estimates for BRCA1/2 carriers. The incident rates against which the women are weighted are derived from population based studies. By this procedure, hazard ratios are typically shifted away from the null value (=1) at the cost of some power (wider 95% confidence intervals).
The physical activity study was conducted before subcohort-specific weight calculation became available. Therefore, this subcohort analysis was performed within the entire cohort, by including a 1-0 variable in the model that indicated whether the time-varying record was to be in- or excluded in the subcohort, using the weights that were determined for the entire cohort. After the physical activity study was published, a method to calculate subcohort-specific weights became available. The body weight study showed an interesting association between body weight and postmenopausal breast cancer risk, however, the analyses were conducted without the weighted regression approach because the power was relatively low and resulted in rather unstable weights. We preferred, nevertheless, to conduct and show the postmenopausal analysis unweighted, taking into account that estimates might be slightly biased towards the null. The subcohort in the diagnostic radiation study and the premenopausal subcohort in the body weight study were large enough to calculate weights for both the entire cohort and subcohort separately.
Nearly all women with breast cancer (and eligible for BRCA1/2 DNA testing based on their strong family history) undergo DNA testing based on their diagnosis, while many unaffected women (eligible for BRCA1/2 DNA testing based on their strong family history) may not (see Figure 1). While the cancer-related testing is accounted for by the weighted cohort approach, the decision to opt for testing may also be related to risk factors for breast cancer. The weighted cohort approach adjusts sufficiently for selection bias as long as the selection of cases and unaffected women is independent of the risk factor under study. If the risk factor selection is, however, differential according to case-status, a weighted analysis might still confer a biased association between the risk factor and breast cancer risk, i.e. the true association among all carriers in the background population might be different. For example, it has been suggested that parous women may be more inclined to opt for testing[11]. A methodological study on testing bias in the HEBON study (Woudenbergh et al., *personal communication*) showed that the association between nulliparity (no/yes) and breast cancer risk differed statistically significantly between untested and tested women ($P_{interaction}$=0.002). The same conclusions could be drawn if, instead of parity, the number of children was examined. A positive association between parity and uptake of genetic testing (see Figure 1) might therefore result in bias away from zero in retrospective studies among BRCA1/2 carriers indentified in the clinical setting. Balmana et al. observed that offspring gender might also affect testing behaviour; among women without breast cancer, women that have at least one daughter, were more likely to opt for testing[12]. However, as gender is not associated with breast cancer risk, testing bias based on offspring gender is not an issue. Brohet et al. observed that women who opt for testing more often had used oral contraceptives[13]. However, this overrepresentation was similar for women with and without breast cancer.

Other personal characteristics found to be associated with breast cancer genetic testing uptake were older age, women of Ashkenazi Jewish heritage, unmarried status, personal breast cancer history, and family history of breast cancer[14]. However, these associations were inconsistent across studies, including personal and family history of breast cancer which failed to show a statistically significant correlation with uptake in some studies and showed an association in the opposite direction in others[14]. Additionally, a psychosocial review showed that some women look with anxiety upon the potential of planning their life in view of a risk, while others believe that only through knowledge and awareness they can improve control of their life[15].

How these previously investigated characteristics and views may potentially relate to healthy behaviour is unknown. To date, there is no literature on testing behaviour based on other risk factors for breast cancer, like physical activity, body weight, and diagnostic radiation, which were the subject of the studies described in this thesis. Assuming that most cases undergo genetic testing because of their diagnosis, testing behaviour of unaffected carriers based on a healthy lifestyle might result in bias away from zero if the healthy behaviour is associated with breast cancer risk. However, healthy behaviour resulting in testing behaviour may also increase the chance of uptake of prophylactic surgery after test disclosure, and as a result, censoring in the statistical analysis. Bias by censoring may occur if censoring is dependent on the risk factor under investigation. However, this may be a problem in future studies only as the number of incident cases is currently low. Assuming the selection mechanisms for both testing behaviour and uptake of prophylactic surgery are the same, the effects of both on the risk estimate is in the opposite direction. For example, the proportion of physically active unaffected carriers may be too high due to testing behaviour but too low due to uptake of prophylactic surgery. Similarly, the proportion of obese unaffected carriers may be too low due to testing behaviour and too high due to lack of uptake of prophylactic surgery. The ultimate direction of these biases is difficult to predict and they may outweigh each other.

Survival bias

Survival bias can occur if the risk factor under investigation is associated with overall survival and longer survival increased the chance of study entry, i.e. the study includes prevalent cases (see Figure 1). If we assume that the risk factor under investigation increases the risk of breast cancer ànd mortality, cases that were exposed to the risk factor are underrepresented when including prevalent cases only, because many exposed cases had died before the study started. An example could be the diagnostic radiation study: prevalent cases who were exposed to a greater extent may not have survived until the start of the cohort. Vice versa, if we assume that the risk factor under investigation is inversely associated with breast cancer risk ànd mortality than cases that were exposed to the risk factor (in this case protective factor) factor are overrepresented. An example may be found in the physical activity study. Both scenarios lead to bias towards unity. However, other scenarios could be that the risk factor under investigation decreases breast cancer risk but potentially increases risk of death or vice versa. Then

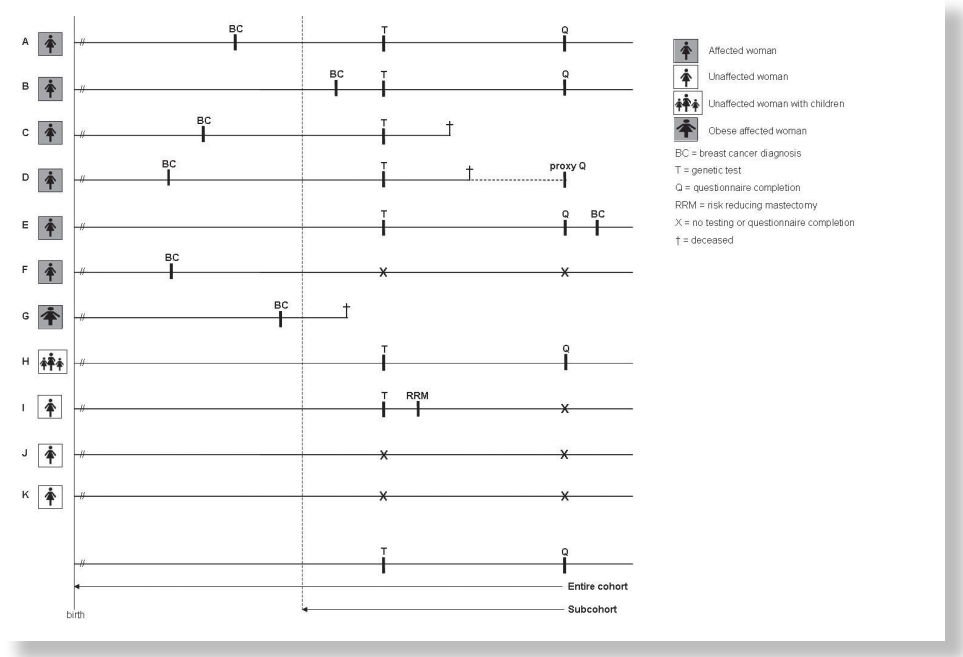

FIGURE 1. Hypothetical selection bias in analyses on a risk factor and risk of breast cancer in the HEBON study. We assume women A to K are all BRCA1/2 mutation carriers and eligible for the study. Testing bias (apart from the testing bias based on case-status which is accounted for by the weighted cohort approach) can occur if the uptake of genetic testing is differential to both the risk factor and the case-status: for example, unaffected woman H opted for genetic testing because she has children; unaffected women J and K (without children) did not opt for genetic testing, whereas among affected women having children is not related to uptake of testing as being affected already directs the uptake of genetic testing. Survival bias can occur if the risk factor under investigation, for example obesity, is associated with overall survival and longer survival increased the chance of study entry, i.e. the study includes prevalent cases; for example, women G died of breast cancer before genetic testing was possible, while women A to D survived until genetic testing and partly until study entry. The different analytical approaches to investigate potential survival bias are indicated at the bottom of the figure: the follow-up in the entire cohort analysis starts at birth, while the follow-up in the subcohort analysis starts at 5 or 10 years prior to questionnaire completion.

the ultimate direction of the bias is difficult to predict and would be determined by the balance between the two counteracting effects.

The ideal study design is a prospective study which does not have the problem of survival bias. However, as explained, at this moment, a prospective study of BRCA1/2 mutation carriers lacks power and this may remain a problem in the future. In the study populations of the diagnostic radiation, physical activity, and body weight studies, there were 11, 14, and 9 incident cases, respectively. Another approach to investigate survival bias is to exclude cases diagnosed a long time ago – so long-term survivors - whose prognosis may have been influenced by the exposure. Therefore, for

each of the three association studies, we performed a subcohort analysis (or: pseudo-incident cohort analysis) in which we only included women who were diagnosed (or for unaffected women censored) within 5 years prior to questionnaire completion (study entry). Because of power issues, in the physical activity and body weight studies, we could only conduct a subcohort analysis restricted to women who were diagnosed (or for unaffected women censored) within 10 years prior to questionnaire completion. The subcohort selection is depicted in Figure 1. To assess whether survival bias was present, we compared the results of the entire cohort and subcohort analyses (see Table 1). In the general population, there is convincing evidence that overweight (BMI between 25.0 to 29.9 kg/m^2) and/or obesity (BMI≥30.0 kg/m^2) decrease survival[16-18] and breast cancer-specific survival[19,20]. Physical activity has also been related to overall survival[21,22] and breast cancer-related survival[23-25]. The evidence for a prognostic value of diagnostic radiation is limited. A recent Dutch study among Hodgkin's lymphoma patients showed that radiation-induced breast cancers have a distinct gene expression profile and may have a less favourable prognostic profile[26]. To our knowledge, this is the only study that investigated radiation as a prognostic factor. However, the exposure concerned was high and not low dose ionizing radiation, such as diagnostic radiation. There are no data on diagnostic radiation, physical activity, and body weight as prognostic factors in BRCA-associated breast cancer. As shown in Table 1, all three studies showed a difference in risk factor associations between the entire cohort and the subcohort in the direction that could have been expected based on potential survival bias as described above. For physical activity, survival bias seemed to have caused bias through the null instead of just towards the null.

Although survival bias seems to be the most likely explanation for the difference between the entire cohort and subcohort analyses, the inconsistency might also partially result from differences in characteristics between the two analytic cohorts (see Table 1). The subcohort is a selection on higher ages at diagnosis (cases diagnosed at a young age have a worse prognosis), a younger age at questionnaire completion, more recent birth cohorts and associated profile of risk factors (e.g. a greater use of oral contraceptives, a smaller number of children, and a higher age at first full term pregnancy). In the general population, there is no evidence that there is an interaction between radiation and physical activity with age. For body weight, menopause is a clear effect modifier of the association with breast cancer risk, hence the stratification on menopausal status is important. Second, methodological studies, including ours, show that validity and reliability of self-report are better with a younger age at questionnaire completion.

To investigate whether differences in age at questionnaire completion (hypothesis based on prediction model test-retest reliability study) could have caused (part of) the differences observed between the entire cohort and the subcohort in the diagnostic radiation study, we stratified both the entire cohort and subcohort analysis according to age at questionnaire completion (split on median 45.5 years). The difference between both analytical cohorts remained approximately the same (data not shown). Also, adding age at questionnaire completion to the model did not affect the risk estimates (data not shown). For all three association studies, the associations that we observed were not limited to recent birth cohorts only nor did we find evidence for interactions between birth cohorts and the risk factor under investigation (data not shown). Additionally, previous studies[13,27,28] similar to ours, did not show consistent differences between their entire and subcohort analyses, also indicating that the differences in characteristics between the entire and the subcohort is likely not to be the only explanation for the observed differences in risk estimates between both analytical cohorts, but that the observed differences depend on the risk factor under investigation. Our observation of stronger risk increases in the subcohort versus the entire cohort analysis in the diagnostic radiation study was in line with the IBCCS X-ray study that showed a similar difference between their two analytical cohorts (i.e. higher risk estimates in the subcohort as compared to the entire cohort), however, this finding was not addressed in the paper[27].

In conclusion, none of the characteristics are likely to explain the observed differences between the entire cohort and the subcohort. Because the subcohort analysis is restricted to relatively recent diagnoses and is much less likely to suffer from survival

TABLE 1. Comparison of risk estimates for breast cancer and characteristics by analytic cohort for each association study

	Diagnostic radiation (chapter 2)		Physical activity (chapter 5)		Body weight (chapter 6)	
	Entire cohort	**Subcohort**	**Entire cohort**	**Subcohort**	**Entire cohort**	**Subcohort**
Pseudo-incident period	not applicable	5 years	not applicable	10 years	not applicable	10 years
N (n cases)	1,993 (848)	1,122 (174)	1,026 (468)	725 (218)	342 (97)	299 (63)
HR, 95%CI[a]	1.39 (1.12-1.73)	1.90 (1.20-3.00)	1.21 (0.94-1.57)	0.84 (0.57-1.24)	1.33 (0.80–2.22)	1.56 (0.85-2.87)
Age at diagnosis[b]	39.5 ± 7.4	42.0 ± 7.1	42.2 ± 10.0	43.7 ± 10.9	38.2 ± 6.5	38.8 ± 7.4
Age at questionnaire completion[c]	45.3 ± 10.5	41.1 ± 9.7	48.2 ± 13.1	45.5 ± 12.7	48.2 ± 13.0	42.1 ± 9.5
% born ≥1960	58%	75%	38%	50%	18%	21%

HR, hazard ratio; CI, confidence interval

a Diagnostic radiation study: ever versus never diagnostic radiation before age 30; physical activity study: ever versus never sports activity lifetime; body weight study: adult weight change and the risk of postmenopausal breast cancer

b In years; mean ± standard deviation

c In years; mean ± standard deviation; proxy questionnaires excluded for the physical activity and body weight studies

bias, the results of the subcohort are considered to be the most valid, although they are based on smaller numbers and therefore less precise (see Table 1). It has been shown that tumours grow quickly in women with BRCA1 mutations and in young women[29]. Depending on the question whether the effect of a risk factor has a direct or a long term influence on prognosis, the possibility of some survival bias in the results based on the subcohort cannot be excluded, especially in the physical activity and body weight studies where the pseudo-incident period was 10 instead of 5 years. Thus, the actual association could even be stronger.
In an attempt to reduce or eliminate potential survival bias a priori, information on deceased (obligate) carriers was collected through proxies for the physical activity and body weight studies. Obligate carriers are not tested themselves but considered as carrier because they had at least one proven carrier among their children, while inheritance was not paternal. In most cases the proxy was a daughter (69%) or a sister (13%) and less often the spouse, a son, the mother, a cousin, or a granddaughter. Unfortunately, a large proportion of proxy questionnaires had to be excluded because of incomplete information, indicating that using a proxy to provide information on physical activity and body weight history is problematic. In the final analyses, only 12% (N=37) and 6% (N=14) of the proxy data of eligible deceased carriers (N=299) was used in the physical activity and body weight study, respectively. By exclusion of a large part of the available proxy data, we did not diminish potential survival bias. Therefore, the subcohort analyses from which most deceased cases were excluded by definition remained a very important approach.

Non-response bias

Among proven BRCA1/2 mutation carriers participating in the HEBON study, the response rates to our mailed baseline and follow-up questionnaire were 80% and 84%, respectively (proxy questionnaires excluded). Similar to studies in the general population, the response rate among breast cancer cases was higher than among unaffected carriers (84% versus 78%, respectively; baseline response rate). The response among proxies of deceased obligate carriers was 76%. The response rate to the diagnostic radiation study questionnaire (sent out together with a standardized follow-up questionnaire) was 76%. These high response rates indicate that carriers are highly motivated to participate in epidemiological surveys and suggest that selection bias due to non-response is not very likely in our studies. Active refusal contributed only approximately 15% to the total non-response. Reasons for refusing participation were lack of time, psychological burden of completing the questionnaire, and being in the middle of cancer treatment and/or recovering from surgery. Similar to the potential selection mechanism described for testing behaviour, we cannot exclude the possibility that women with a relatively healthy lifestyle and women undergoing screening have been more willing to participate which could have slightly affected our risk estimates.

Missing data

In the physical activity and body weight studies, we excluded women with >50% missing data. The remaining missing values in the physical activity and body weight study, as well as missing values in the diagnostic radiation study were included as a separate category. Missing data in the physical activity and body weight studies primarily originated from proxy questionnaires and were excluded, while in the diagnostic radiation study all questionnaires were completed by the carriers themselves. For all three association studies, missing values in ever/never exposure were included as a separate category. In the diagnostic radiation study, among carriers with any exposure to diagnostic radiation in a certain age period, missing values for age at first and number of exposures were imputed by age period, using the average age and number of exposures of women for whom complete data were available. Restricting the analysis to subjects with complete data on the variables involved in the analysis can result in biased estimates, especially if the subjects who are included in the analysis are systematically different from those who were excluded in terms of one or more key variables. Women who were excluded from, for example, the body weight study, did not statistically significantly differ from women included in the study in characteristics like percent cases (50% and 46%, respectively) and BRCA1 carriership (78% and 79%, respectively). For diagnostic radiation, the proportion of missing values in ever/never exposure varied according to the type of exposure (1.6-10%). A sensitivity analysis on ever versus never exposure to diagnostic radiation before age 30 excluding the missing category resulted in a similar association as when including the missing category in the analysis (HR=1.78 (1.23-2.55) and HR=1.73 (1.20-2.48), respectively; unweighted analysis in the subcohort). In summary, the inclusion or exclusion of missing data is not likely to have resulted in selection bias.

MISCLASSIFICATION BIAS

Outcome assessment

Information on cancer history and prophylactic surgery was either collected through Netherlands Pathology Database (PALGA) (breast and ovarian tissue related records only)[30] and the Netherlands Cancer Registry (NCR) (cancer history only) until August 2007 and/or self-reported (questionnaire) for the period not covered by the registries (<1989). Linkage was only possible for whom permission was obtained (97%).
The first primary invasive breast cancer or ductal carcinoma in situ (DCIS) was the main outcome of interest. Five percent of breast cancer diagnoses were DCIS. We considered DCIS as breast cancer although it is unknown whether all lesions would have developed into invasive carcinoma. However, it has been shown that there is a high prevalence of DCIS adjacent to BRCA-associated breast cancers[31] and in prophylactically removed breasts of BRCA1/2 mutation carriers[32,33], indicating that carriers are prone to develop epithelial lesions that confer a high risk of subsequent invasive breast cancer. DCIS is equally prevalent in patients who carry deleterious BRCA mutations as in high familial-risk women who are non-carriers, but it occurs at an earlier age[34]. Hwang et al. also

observed that high-grade DCIS was more common in BRCA1 mutation carriers than in non-carriers. Furthermore, DCIS is a frequent finding because of the relatively high frequency of screening in carriers. These studies argue for the consideration of DCIS as breast cancer in BRCA1/2 mutation carriers.
The risk factor questionnaires were all completed between 1999 and 2007. With the 10 year pseudo-incident period in the subcohort analyses of the physical activity and body weight studies, the range of calendar years of diagnoses was between 1989 and 2007. As a result, by linkage with PALGA and NCR, the coverage of confirmation of diagnoses and prophylactic surgery in the subcohort analyses was higher than for the entire cohort. Self-reported breast cancer is generally valid; Manjer et al. reported a 97% confirmation rate according to the Swedish Cancer Registry[35]. In our studies, 96% of breast cancers diagnosed after 1988 were confirmed by PALGA/NCR. Four percent was not validated, mainly due to carriers diagnosed and treated abroad and likely errors in linkage data.

Recall bias

Diagnostic radiation, physical activity, and body weight were all self-reported in these studies. Inaccuracy of self-reported exposure may lead to non-differential and/or differential misclassification which would affect the risk estimates in different ways: non-differential misclassification (inevitable in any study using subjects' information only) mostly results in bias towards unity. However, in case of variables with more than 2 categories the result of possible non-differential misclassification of exposure is difficult to predict, and may be away from the null for one of the categories[4]. Inaccuracies that are systematically different in breast cancer cases and unaffected individuals (differential misclassification or recall bias) can either mask true associations or create spurious ones. Recall bias can arise if cases, because of their disease diagnosis, systematically overreport or underreport their exposure compared with controls. We had expected a priori a certain amount of non-differential, and possibly differential, misclassification for early life exposures, such as radiation exposure before age 20. These exposures, especially diagnostic radiation, before ages 6-10 would probably be difficult to remember and might therefore not have been reported anyway. Still, both cases and unaffected carriers had reported to have had chest fluoroscopies and X-rays before age 8 (N=24 (14 cases and 10 unaffected carriers) and N=52 (19 cases and 33 unaffected carriers), respectively). And in the physical activity study, 196 carriers had reported some sports activity before age 8. In the body weight study, only exact weight at age 18 and weight at older ages were reported and we did not ask to report weight during childhood.
Recall bias could occur if cases misremembered behaviours consistent with publicized exposure-disease associations or if lifestyle changes following diagnosis affected their memory of past behaviours. We hypothesized that, in our studies, recall bias may be less of a problem than would be expected based on a study population of women from the general population, as clinically tested unaffected BRCA1/2 carriers may be considered to be more health conscious than unaffected women in the general population, because they belonged to a breast and/or ovarian cancer family and opted for genetic testing.

As expected, we observed that accuracy of self-reported mammograms in carriers was sometimes better than in the general population, but not exceptionally high. An explanation may be the relatively high frequency of mammographic screening among carriers; Pogoda et al. observed that greater total exposure was associated with greater disagreement[36].
Because of the important clinical implications of the diagnostic radiation study (chapter 2) and the issue of recall bias that has been raised as an important limitation of previous studies on this subject, we performed two methodological studies investigating whether non-differential and differential misclassification between cases and unaffected carriers exists. Both the test-retest reliability study (chapter 3) and the validity study (chapter 4) indicated that inconsistent self-report of diagnostic radiation by BRCA1/2 mutation carriers was mainly non-differential by case-status. This finding is in agreement with two other validation studies which showed a certain amount of disagreement between self-reported prediagnostic mammography and medical records but no differences between cases and controls[36,37]. Accuracy of self-report of other types of diagnostic procedures, was investigated by Pogoda et al.[36] and Berrington de Gonzalez et al.[38]. Similar to what was observed in the mammography validation studies, they observed overreporting of frequently-occurring events but they found that reporting errors by cases and controls were largely non-differential. Berrington de Gonzalez et al. also compared risk estimates based on self-reported data on diagnostic X-rays and medical record data and observed no large differences[38]. The recent review and meta-analysis by Howard et al. on the validity of self-reported mammography among women from the general population did not investigate differences between cases and controls[39].
The implications of the results of the methodological studies on the risk estimates of the diagnostic radiation study, is discussed in a separate section of this chapter (page 146).
As explained in chapter 5, recall bias as an explanation for the observed inverse association between sports activity and breast cancer risk does not seem likely, because as shown in the Nurses' Health Study[40], cases tended to underreport their activity less than controls, which would result in an underestimation of the protective effect of physical activity on breast cancer risk. An objective measurement, i.e. with an activity measuring device, of lifetime physical activity is clearly not feasible. Although our questionnaire was based on a validated questionnaire[41], measuring lifetime physical activity is difficult and recall of physical activity in the distant past may be more difficult than recalling recent activities[42]. Therefore, it is probable that a difference between cases and unaffected carriers in detail of reporting sports activity in different age periods has influenced the risk estimates of the association between sports activity and breast cancer risk to some extent. We observed a statistically significant risk reduction for ever participating in sports activity after age 30 while for sports activity before age 30, we only observed risk reductions among active women. Additionally, we found statistically non-significantly increased risks for the lowest categories of all three measures of sports activity when compared to no sports activity before age 30. Cases might be more motivated to report relatively short periods of sport activity in the distant past (or at relatively young ages) than unaffected carriers while for recent activity, quality of recall may be more equal for both groups.

Although reported and objective weights are correlated[43], studies on the validity of self-reported anthropometric measures show consistent underreporting of self-reported body weight and overreporting of height, especially among overweight and obese individuals[43,44]. We are not aware of studies that examined potential differential misclassification according to breast cancer case-control status. However, even if we assume that misclassification is non-differential, the systematic underreporting of obesity might have resulted in an overestimated risk for the one to highest category of BMI (22.5-25.0 kg/m^2) in the premenopausal cohort.
In summary, misclassification bias in the diagnostic radiation, physical activity, and body weight studies was largely non-differential and will most likely result in relative risks biased toward the null.
Whether the differences in results between the entire cohort and subcohort analysis in the physical activity and body weight studies are a result of survival bias (described previously in this chapter), a difference in non-differential and/or differential misclassification, or a combination, is difficult, if not impossible, to disentangle without objective verification of the exposure and further studies on prognostic factors of survival in BRCA1/2 mutation carriers. The differences between the entire cohort and subcohort results are likely to be due to a combination of survival bias and non-differential misclassification for several reasons. Misclassification of self-reported information on diagnostic radiation history was mainly non-differential and accuracy of self-report of exposure in recent periods was better than for periods longer ago and better for younger women, however, as described previously, the risk estimates were not different by age at questionnaire completion.

Missing data

In the diagnostic radiation study, missing values in ever/never exposure (varying between 1.6% for mammograms and 10% for fluoroscopies) and covariates (<1%) were coded as an additional category (as described in one of the previous paragraphs). Missing values for age at first exposure, age at last exposure, and number of exposures were imputed only if carriers were known to have been exposed to a certain procedure in a certain age period as follows. Missing values for age at first (<15% for fluoroscopies and X-rays, and <10% for mammograms) and last exposure (<5%) and number of exposures (<21% for fluoroscopies and X-rays, and <7% for mammograms), were imputed by age period, using the average age and number of exposures of women for whom complete data was available. For example, based on known data of more than 450 carriers, the missing age at first X-ray before age 20 of 85 carriers was imputed by 14.1 years and the missing number of X-rays before age 20 of 120 carriers was imputed by 2.23 X-rays. Thus, we assumed that women with missing values for number of exposures had had more than 1 procedure. This may slightly have affected our risk estimates, but the direction is predictable, i.e. as a result of this imputation method, in which we did not distinguish between cases and unaffected carriers, risk estimates are probably somewhat attenuated. With regard to the cumulative breast dose score, if at least one out of four procedures (i.e. ever/never fluoroscopies, X-rays, mammograms, and/or CT-scans) was missing, then the dose score was set to missing. This resulted in a proportion of missing dose score of 18% (350/1,993). To conduct a sensitivity analysis, an imputed cumulative dose score was calculated as follows. Missing values in ever/never exposure to mammograms, CT-scans, and for the UK and France fluoroscopies, were set to never exposure. Missing values in ever/never X-rays were imputed with age and calendar specific number of exposures.
For the Netherlands, missing data in fluoroscopies were also imputed with age and calendar specific number of exposures as in the Netherlands young people were screened for tuberculosis by fluoroscopy between 1940 and 1960 through a population-based screening program[45] (see also section on dosimetry later in this chapter).
As expected, analysis of the imputed dose score resulted in an attenuation of the risk estimates (data not shown) with a similar conclusion as when the missing data category was included as a separate category. Analysis of the cumulative dose score excluding the missing data category did not materially affect the risk estimates (data not shown).

Confounding

A confounder is a factor that is associated with the exposure and independently affects the risk of developing breast cancer. If the prevalence of these confounding factors differs between cases and unaffected women, they will distort the observed association between the disease and exposure under study if not taken into account. All statistical analyses were based on multivariable models, i.e. adjusted for confounding factors. Forward stepwise confounder selection, in which the effect of adding one confounder at a time was evaluated, was based on a more than 10% change in (at least one of) the estimates of the main exposure variables. As data on all potential risk factors for breast cancer was available from our detailed questionnaires, it is unlikely that our results are explained by confounding by known risk factors of breast cancer.
Residual confounding might be a problem in the subcohort analysis of the physical activity study, as this analysis was performed within the entire cohort, using confounders determined on testing in the entire cohort. The physical activity analyses were adjusted for BMI at age 18 and age-specific BMI, while the body weight analyses were adjusted for age-specific mean MET hours per week of lifetime sports activity. The literature describes a limited confounding effect between the two variables[46,47]. Because of mutual relevance we decided beforehand to take BMI into account in the physical activity analyses and vice versa, independent of whether they fulfilled our confounder selection criteria. In concordance with the literature, BMI was a minor confounder in our physical activity study (max 5% change in estimate of ever/never sports activity). Furthermore, we observed no strong evidence for effect modification by BMI although the inverse association between sports activity and breast cancer risk was somewhat stronger in relatively lean women, this was not statistically significantly different from the risk estimates for relatively heavy women ($P_{interaction}$>0.05). In the physical activity study we can, however, not exclude potential residual confounding by menopausal status-specific BMI. According to our definition of a confounder (i.e. >10% change in estimate), sports activity was a confounding factor for adult weight change in

the body weight study, however, the changes in estimates were small, i.e. 13 and 15% for pre- and postmenopausal breast cancer, respectively.
The risk estimates of the physical activity and diagnostic radiation studies were not materially affected by including the estimates for several age periods in the same model, or adjustment for other types of exposures. In the body weight study, additional adjustment for height in the models of adult and relative weight change or calculating relative weight change by dividing adult weight change by BMI at age 18 instead weight at age 18, did not affect the results. Additional adjustment for age at menopause in the postmenopausal body weight analyses did not result in a different conclusion either.
In conclusion, it is unlikely that confounding has had more than a minor effect on our risk estimates.

Familial clustering

Another statistical feature applied in the association studies is the clustering on family to account for potential within-family correlations in risk factors and breast cancer risk. This results in adjustments of the variance of the coefficients by a robust variance estimator. In general, clustering resulted in a maximum of 2.5 to 4% change in variance in the diagnostic radiation study and physical activity/body weight study, respectively, indicating that the effect of familial clustering was marginal. The proportion of clusters (i.e. clusters/carriers) in the different studies was 83% (930/1,122), 56% (404/725), and 54% (326/609) in the diagnostic radiation, physical activity, and body weight (premenopausal) study, respectively.

STRENGTHS

Despite the methodological considerations and limitations described above, there are also a number of strengths of the studies described in this thesis. Strengths of the studies on the association of breast cancer risk with diagnostic radiation, physical activity, and body weight include the large sample sizes, the detailed information on exposure in different age periods, medical confirmation of nearly all breast cancer diagnoses, the high response rates, the weighted cohort approach, and the time-varying analyses. Additionally, the infrastructure of the Netherlands health care and the existence of national registries like PALGA and NCR resulted in complete case ascertainment for the entire study period.
More specifically, the diagnostic radiation study was a collaboration between three large BRCA1/2 mutation carriers cohort studies, i.e. GENEPSO (France), EMBRACE (UK), and HEBON (the Netherlands). A detailed questionnaire on lifetime diagnostic radiation history was especially developed for this study. The questionnaire contained indication-based questions on lifetime exposure to fluoroscopies, chest/shoulder X-rays, mammograms, chest/shoulder CT-scans, other diagnostic procedures using ionizing radiation (e.g. bone-scan) involving the chest and/or shoulders, and radiotherapy. This questionnaire was also tested in a pilot study in the UK and the Netherlands. Additionally, a unique feature of this study was the individually estimated cumulative

breast dose score. This score was based on exposure to fluoroscopies, X-rays, mammograms, and CT-scans and was calculated by the sum of the age-specific number of procedures multiplied by nominal estimates of breast dose. For this dose score calculation, a literature review, including review of historical literature on tuberculosis screening in the Netherlands, and several discussion sessions were specially conducted to obtain the estimates of the breast dose. More details can be found in the dosimetry paragraph in the next section. Moreover, the most important strength was the fact that we conducted two methodological studies to investigate potential (differential) misclassification bias. Furthermore, we investigated potential bias by family history, i.e. self-selection to have early mammography by carriers based on a close relative with early breast cancer.
The test-retest reliability study and the validity study are the first studies investigating reliability and validity of self-reported risk factor exposure information in BRCA1/2 mutation carriers. Moreover, these are also among the first studies assessing accuracy by case-status to investigate potential recall bias. Additional strengths include the assessment of reliability of more than one diagnostic procedure, reliability/validity of age at first exposure, and investigation of reliability/validity of prediagnostic mammograms, although the numbers for the latter were sometimes small.
An important feature of the physical activity and body weight studies was the adjustment for each other. Therefore, the observed associations were mutually independent. In the body weight study, we have stratified the analyses according to menopausal status, which is a well-known effect modifier in the general population.

DIAGNOSTIC RADIATION STUDIES

GENETIC SUSCEPTIBILITY, IONIZING RADIATION, AND BIOLOGICAL MECHANISM

As previously described (chapter 1), BRCA1/2 mutation carriers might be more susceptible to radiation-induced breast cancer than non-carriers, or the general population due to impaired DNA repair mechanisms. A few studies have examined whether BRCA1/2 mutations modify the risk of breast cancer from diagnostic radiation. Two studies[27,48] have reported an association between self-reported exposure to chest X-rays and breast cancer risk in BRCA carriers. Risks were particularly high among those exposed before age 20, and observed at dose levels considerably lower than at which increases have been found in other radiation-exposed cohorts that did not rely on self-reports: the predictive relative risk (based on the model of Preston et al. where a RR of 2.0 for a dose of 1 Gy assuming an age at exposure of 25 years[49]) for a dose of 20 mGy before age 30 is 1.02, which is substantially less than the lower 95%CI in the IBCCS[27] and our diagnostic radiation study (chapter 2). The IBCCS X-ray study[27] reported a 1.8-fold increased risk for ever versus never exposure to X-rays in carriers in the subcohort while we observed a 1.4-fold statistically non-significantly increased risk for exposure to X-rays before age 40 (HR=1.38, 95%CI=0.87-2.20). This difference may be explained by the fact that we included prediagnostic X-rays only while the IBCCS had included all X-rays lifetime. However, differences in design and questionnaire format make it difficult

to compare these results. Analyses in the overlapping group (417 (21%) participants) lacked power. Our diagnostic radiation study also showed that breast cancer risk was increased after exposure at relatively young ages (i.e. before age 30) and that there was no association with exposures at ages 30-39. These findings confirm the evidence suggesting that breast tissue might be more susceptible to carcinogenic influences during early life and adolescence because breast tissue is not fully differentiated until after the first full-term pregnancy[50]. However, the risk of breast cancer in carriers exposed before age 20 was not more strongly increased than among those exposed at ages 20-29, in contrast to what was observed in other radiation-exposed cohorts that did not rely on self-reports[49,51]. As described previously in this chapter (see paragraph on recall bias), exposure before age 20 may have been more prone to non-differential misclassification than exposure at ages 20-29, resulting in bias towards unity. Two other carrier studies[52,53], focussing on mammography exposure and breast cancer risk, did not observe an association. The unmatched case-control study by Goldfrank et al. was relatively small, women had a median age at first mammogram of 35 years, and it is not clear if and how many BRCA1/2 mutation carriers had had a mammogram before age 30[52]. Narod et al. conducted a large matched case-control study in which the mean age at diagnosis was 40.5 years and the mean age at first screening mammography was 35.3 years[53]. Still, 201 cases had had their first mammography before age 31, however, there was no associated with risk of breast cancer (OR=1.00, 95%CI=0.78-1.29)[53]. Similar to our diagnostic radiation study, only prediagnostic mammograms were considered. The mean age at diagnosis in our diagnostic radiation study was also very comparable (39.5 years, see Table 1). In our entire cohort analysis (with follow-up starting at birth), we also observed no association between mammogram exposure before age 30 (HR=1.05, 95%CI=0.86-1.30 (Table 4, chapter 2)). However, we observed a 1.5-fold increased risk of breast cancer after mammogram exposure before age 30 in the subcohort, an analytical approach to investigate potential survival bias that was not performed by Narod et al.. We were concerned that family history of breast cancer at an early age may be an indication for mammographic screening at a young age, resulting in potential bias away from the null value. However, subgroup analyses in carriers who never had a mammogram before age 30 showed that our finding of an increased risk of breast cancer after early mammography did not seem to be attributable to confounding by family history. Several other studies examined whether the risk for breast cancer from low dose radiation was modified by polymorphisms in several other DNA repair genes, but were too small and require replication to substantiate the findings[54,55].

A puzzling finding was a difference in the association between cumulative dose score and breast cancer risk for BRCA1 and BRCA2 carriers in the entire cohort and subcohort (chapter 2, Table 7). For these age-specific exposure effects the power in the BRCA1/2 groups is rather low and random fluctuation by non-differential misclassification is the most likely explanation for the observed differences. However, we cannot exclude the possibility that the effects of ionizing radiation on breast cancer risk differ between BRCA1 and BRCA2 mutations. BRCA1 and -2 proteins both play a role in the repair of DNA damage of double strand breaks, which can be caused by ionizing radiation.

However, their functions are quite distinct as germline mutations in the two genes predispose to different age-specific risks of breast cancer and to different subtypes of breast cancer. To our knowledge, there are no studies indicating that BRCA1 and BRCA2 carriers respond differently to damage caused by ionizing radiation, low or high dose. In vitro studies failed to find an association between heterozygous BRCA1/2 mutations and defects in the ability to rejoin radiation-induced DNA breaks[56]. So far, for higher (therapeutic) radiation exposures from radiotherapy no increased risk for contralateral breast cancer in carriers was observed, but the average age at diagnosis and radiotherapy exposure in these studies was >40 years, and these studies were somewhat underpowered and not designed to answer this research question[57,58]. Broeks et al. reliably assessed potential gene-radiation interaction in a case-only study in women with contralateral breast cancer after a first breast cancer diagnosed before age 50 and showed that the risk of radiotherapy-induced BC was increased 2.5-fold for carriers of a mutation in a DNA Damage Repair Pathway gene (BRCA1, BRCA2, CHEK2, or ATM) versus non-carriers[59]. The WECARE study observed that carriers of a mutation in the CHEK2 gene who received radiotherapy for their first breast cancer, compared with non-carriers not treated with radiotherapy, had an relative risk of 2.6 of developing contralateral breast cancer, however, they observed no statistically significant association between the mutation and contralateral breast cancer overall or among those treated with radiotherapy[60]. Larger studies will be required to determine whether a difference exists in response to ionizing radiation between BRCA1 en BRCA2 carriers. As a couple of studies appear to reveal some differences in the biological responses to high- and low-dose radiation in the general population[61], future studies should also address whether this difference in response is modified in BRCA1/2 mutation carriers.

Our follow-up may not have been long enough to detect an association between radiation exposure at ages 30-39 and breast cancer risk as the average age at end of follow-up was 39 years and in the general population the induction period for breast cancer after exposure to radiation is estimated to be at least 8 years and on average 10 to 15 years[49]. Since we hypothesized that BRCA carriers may have increased radiosensitivity due to impaired DNA repair mechanisms, we used a 5 year time lag in our analyses. Analyses using a 2 or 10 year time lag showed very similar results (data not shown). We kept the 5 year time lag to minimize potential contamination by diagnostic procedures related to breast cancer diagnosis and exclusion of radiation dose that probably did not contribute to induction of breast cancer.

In the general population, radiation-associated breast cancer risk appears to be modified by factors related to timing of reproductive events, such as age at radiation exposure, parity, age at first live birth, and age at menopause[51]. However, these data are based on singular relatively high dose exposures. It was not possible to investigate this in our study of protracted low dose ionizing radiation exposure. A recent study within the US radiologic technologists cohort found suggestive evidence that common variants in selected estrogen metabolizing genes may modify the association between relatively low dose exposure (i.e. occupational and personal diagnostic ionizing radiation exposure) and breast cancer risk[62]. It would be interesting to investigate this in BRCA1/2 mutation carriers as well, at least for age at radiotherapy and risk of contralateral breast cancer.

DOSIMETRY

The individually estimated cumulative breast dose score was a unique feature of the diagnostic radiation study. However, the assumptions underlying the calculation of the cumulative dose score may have introduced some bias in our results. The dose score, which resembled adsorbed dose, was for the largest part based on published dosimetry studies. Dose of X-rays and CT-scans were based on published data by Sigurdson et al.[63]. Mammogram dose was estimated by a review of relevant literature from the 20th century (Thierry-Chef et al, *personal communication*). A representative value of dose to the breast was derived for each publication as well as a weighting factor that reflected the degree of detailed information. Distributions of possible dose received by time period were derived by weighting the available data. Radiation absorbed dose to the breast from a single view in mammography is presently about 2 mGy, about one-fourth the dose in the 1960s-1970s when it averaged about 8 mGy. Because this review included US publications only, we reviewed the European literature ourselves and retrieved reliable dose estimates for the UK and the Netherlands for 1990 onwards. We used the average dose estimates of the UK and the Netherlands to calculate the dose estimates for France.
The most problematic dose to estimate was that of fluoroscopy. Considerable effort has been made on searching information on the breast dose of fluoroscopy for tuberculosis screening, a procedure that was primarily relevant to the Dutch carriers as in the Netherlands young people were screened for tuberculosis by fluoroscopy between 1940 and 1960 through a population-based screening program[45]. Unfortunately, no organ dose estimates for fluoroscopy based tuberculosis screening in the Netherlands were found. Next to reviewing the published literature on the Massachusetts and Canadian fluoroscopy and related studies, we tried to obtain information from the company that manufactured the fluoroscopy and X-ray machines (Delft Instruments), but this provided no useful information because, if there was any information, their archives had been destroyed when the company moved to a new location. Furthermore, we interviewed the chairmen of the historical committee on radiation and the committee on radiation hygiene (Radiological Society of the Netherlands) and we interviewed several retired radiologists on their personal and professional experience with fluoroscopy. The fluoroscopy dose estimates were ultimately determined using the posterior-anterior dose estimate of the Massachusetts and Canadian fluoroscopy studies, a series of papers in the Dutch literature which discussed the abandonment of fluoroscopy for tuberculosis screening based on radiation exposure concerns, and the 10-fold higher dose of fluoroscopy versus X-ray dose as estimated by the experts interviewed.
As fluoroscopy and X-ray procedures may have some resemblance to the patient, this may have created a problem in the reporting of these procedures. Women may have misclassified the reporting of these procedures especially for the period surrounding 1958 because around that period there was a switch from the use of fluoroscopies to X-rays for tuberculosis screening in the Netherlands. This misclassification is likely to be non-differential as both cases and unaffected carriers reported to have had fluoroscopies and chest X-rays at very young ages (see page 136). To prevent as much misclassification as possible, each section of the radiation questionnaire provided a detailed description of the procedure. The fluoroscopies for tuberculosis screening that were reported to have occurred after 1958 were considered as such because fluoroscopies for tuberculosis screening were still performed after 1958 in the case of suspected tuberculosis. Additionally, no indication-specific dose estimates for fluoroscopies and X-rays were used. However, the majority (>95%) of fluoroscopies before age 20 were chest fluoroscopies for tuberculosis screening and originated from the Dutch cohort because of the population-based screening. For X-rays before age 20, the majority (>90%) were chest X-rays for which the dose (0.0005 Gy) differed from the dose of a shoulder X-ray for exposures before 1974 only (0.001 Gy)[63].
Occupational exposure to low dose ionizing radiation has been associated with breast cancer risk in the US radiologic technologists cohort study and may be modified by certain polymorphisms[63-65]. We categorised occupational exposure based on a combination of dose (low or high, based on type of occupation) and duration (short or long) of occupational exposure (low: low dose and short duration, medium: low dose and long duration or high dose and short duration, high: high dose and long duration). We observed no statistically significant association between occupational exposure and breast cancer risk in BRCA1/2 mutation carriers. However, our data on occupational exposure were limited, relied on several assumptions, and the number of occupationally exposed carriers was small. For example, some women reported to have worked as a nurse, but occupational exposure for a nurse can vary widely by work field. Additionally, we imputed part of the data on the age at start and duration of reported occupational exposure, since this information was missing for a large part of the subjects from the UK and France. Also, in the Netherlands, the radiation questionnaire did not include an occupational exposure section, so for Dutch carriers occupational exposure was retrieved from the physical activity section where occupational history was reported; occupations were selected based on the occupations mentioned by the French and English participants. For the same reasons, estimation of dose score for occupational exposure was not an option. It would be very interesting if future studies could investigate the potential association between occupational exposure to ionizing radiation and breast cancer risk in carriers as an increased risk could have major implications for the choice of occupation in BRCA1/2 carriers.
The contribution of the four diagnostic procedures to the cumulative (i.e. until age 40) dose score were 54, 33, 10, and 4% for mammograms, fluoroscopies, X-rays, and CT-scans, respectively. For cumulative dose before age 30 this distribution was quite similar, i.e. 49%, 37%, 10%, and 4%, respectively. The average cumulative breast dose score was 0.014 Gy and ranged from 0.0005 to 0.613 Gy with an interquartile range of 0.002-0.017 Gy. For comparison, the individual annual cosmic radiation dose in the year 2000 in the Netherlands was 0.28 mSv (=0.00028 Gy) which equals approximately half a chest X-ray[66]. The estimated breast dose score from a 2-view mammogram is nowadays 3.5 mGy (0.0035 Gy) (see Table 2, chapter 2). The mammogram doses used are typical doses for an average woman but there are large variations depending on several factors including the characteristics of the patient (breast size, shape, breast composition), parameters of the X-ray machine (kVp, mA, exposure time, target/filter combination and

Half Value Layer, film type and technology) and conditions of exposure (characteristics of the protocols and repetition of exposure in case of poor image quality). Therefore, the dose can increase up to 22 mGy (0.022 Gy) per visit in the 0.1% of women who have large dense breasts[67]. Because carriers are screened from a relatively young age onwards and breast tissue has a higher density at young ages, this may result in more than one set of 2-view mammograms per visit and adjusted parameters of the X-ray machine, resulting in a higher absorbed dose. Therefore, our mammogram dose score may underestimate the true dose, and, as a consequence, the risk estimates presented may correspond to a higher dose category. Sensitivity analysis of the cumulative dose score excluding women exposed to mammograms did not materially affect the results (see Table 5, chapter 2).

BARRIERS IN MEDICAL RECORD REVIEW VALIDATION STUDY

Although medical record data are considered to be the gold standard in self-report validation studies, we came across several barriers while conducting the validation study on self-reported mammograms. Firstly, a number of medical records were missing or did not report on mammograms (17%, 38/218). Records could have been misfiled, lost or destroyed as we were sometimes looking for mammograms taken in the distant past. Under Dutch privacy law (Wet Geneeskundige Behandelins Overeenkomst (WGBO) code 7;545), medical files should be kept up to 15 years after diagnosis. After 15 years they must be destroyed, unless the physician has clinical reasons not to do so. In academic centres, some data in the medical files, such as pathology reports and surgery reports, must be saved up to 115 years. However, the WGBO privacy law was introduced on April 1, 1995 and, in practice, old files from before that time were mostly destroyed. Additionally, medical record notes on previous mammograms taken in other hospitals may have been self-reported by women themselves. Also, women may be more likely to forget to mention a screening test not done at their main site of care to us or to the physician at the time of the questionnaire completion or when giving a medical history, respectively. Records may be incomplete because some hospitals maintained or accessed computerized records only. Considering that overall we could validate 81% of the eligible group and there were no differences between the eligible and validated group (including no difference between cases and unaffected carriers), we believe that these problems have not markedly influenced our results. Nevertheless, among the validations considered successful, we cannot be certain that records were indeed complete. As a result, accuracy may sometimes have been underestimated, especially for items like age at first mammogram if this had occurred a long time ago.

IMPACT OF VALIDITY AND RELIABILITY STUDIES ON THE ASSOCIATION STUDY

Validity is an expression of the degree to which a test is capable of measuring what it is intended to measure. A measurement is valid if its results correspond to the true characteristic. A high reliability (or repeatability) of the measurements does not ensure validity since they may all be far from the true value.
Both the validity study and the test-retest reliability study showed that misclassification of self-reported exposure to diagnostic radiation was largely non-differential by case-

status. However, the validation study arose some concern of potential differential misclassification in the reported age at first prediagnostic mammogram which was underestimated by cases more frequently than by unaffected carriers. In the diagnostic radiation study (chapter 2), the exposure that showed the strongest association with breast cancer risk in carriers was exposure before age 30. Because this measure was based on an age-cut off point, part of the association could have been caused by the potential differential misclassification, if real. Unfortunately, our validation study lacked power to investigate the accuracy of prediagnostic mammograms before age 30. However, if we conducted exactly the same validity analysis on age at first prediagnostic mammograms as reported in the baseline questionnaire (data not shown), the direction of inaccuracy on age at first prediagnostic mammogram was similar in unaffected carriers and cases (i.e. more often a younger self-reported age) indicating that the direction of inaccuracy among unaffected carriers is less consistent than among cases and the presence of differential misclassification depends on measurement time and instrument. Studies with more power are needed to confirm the small underreporting by cases and the more variable pattern among unaffected carriers. Based on our limited data, the differential misclassification that was found, but not replicated, is likely to be small. Furthermore, other findings using pre- and postdiagnostic mammograms also suggested that the evidence for differential misclassification was weak and highly dependent on the size and type of data used: the difference in the direction of inaccuracy of ever/never having had a mammogram before age 30 was not statistically significantly different between cases and unaffected carriers, case-status was not a statistically significant predictor of inaccuracy nor for direction of inaccuracy of age at first mammogram lifetime in the multivariate prediction model, and accuracy of age at first mammogram within 1 year was good and not different between cases an unaffected carriers. Additionally, our gold standard (i.e. medical record information) may not be 100% considering the practical barriers encountered. The two previous case-control studies that investigated accuracy of prediagnostic mammograms only included exposures within 5 years[37] and 10 years[36] prior to diagnosis an did not take age at first exposure or exposure at young age into account. Therefore, we could not compare our results on accuracy of age at first mammogram with data from others.
Assuming that misclassification was mostly non-differential, our finding of a 1.5-fold increased risk of breast cancer after exposure to mammograms before age 30 may therefore be an underestimation of true risk. A sensitivity analysis of the cumulative dose score excluding carriers with mammogram exposure did not result in a different association. Of course, the cumulative dose score analysis may also be an underestimation of true risk as a result of a mixture of non-differential, and possibly differential, misclassification in the other self-reported diagnostic radiation procedures.
A breast cancer diagnosis or among unaffected carriers a risk reducing mastectomy (RRM) and/or a bilateral prophylactic (salpingo-) oophorectomy (BPSO) are life events which may cause greater underreporting of age at first exposures, but only if the exposure is related to the life event, like mammography[68]. As other procedures are not directly related to these life events, recall bias in other diagnostic exposures is less likely

to have occurred. Furthermore, if we assume that exposures, especially diagnostic X-rays, before ages 6-10 would probably not have been reported anyway, the risk estimates presented may correspond to a higher dose category, which may counterbalance the potential underestimation of risk by the non-differential misclassification. However, as described previously in this chapter, there were several women who had reported to have had X-rays before age 8 suggesting that the effect of non-differential misclassification is more important. In case of variables with more than 2 categories, like e.g. number of mammograms, the result of possible non-differential misclassification of exposure is difficult to predict, and may be away from the null for one of the categories.
A more efficient strategy to investigate potential recall bias would probably have been to validate the early mammograms included in the entire diagnostic radiation study population, so in all Dutch participants, and in France and the UK as well. However, unfortunately, the timing of the studies did not allow conducting the diagnostic radiation study before the methodological studies. It would also be worthwhile to put efforts in validating the self-reported X-rays and CT-scans that were included in the entire diagnostic radiation study population. When compared to the study population of the validation study, the diagnostic radiation study population was somewhat younger at questionnaire completion. Therefore, the information in the GENE-RAD-RISK study is likely to be more reliable, as a younger age at questionnaire completion was a determinant of inaccuracy, and retrieving medical records from the distant past may be less problematic than in the validation study. Another interesting option to use for the validation of self-reported exposure to diagnostic radiation in BRCA1/2 mutation carriers could be retrospective biodosimetry[69]. This type of independent verification, which implies linking personal cumulative exposure to radiation to increased frequencies of chromosome translocations, has been used in the U.S. radiologic technologists cohort study[70] and in a recent study among airline pilots[71].
The results of the validity study and the test-retest reliability study indicate that memory is a complex process and that questionnaire development and validation of self-reported exposure are important. Although our questionnaire was not validated, it contained detailed questions and descriptions of the procedures and possible indications to enhance memory and was especially developed for the diagnostic radiation study and adjusted after pilot studies in the Netherlands and the UK. It would be interesting to further investigate which cues or signposts BRCA1/2 mutation carriers used to remember exposure and how they affect measurement error.

PHYSICAL ACTIVITY AND BODY WEIGHT STUDIES

BIOLOGICAL MECHANISM

The biological mechanisms by which physical activity and body weight affect breast cancer risk in the general population has been described in chapter 1. In brief, the main proposed mechanism is through hormone-related pathways; other postulated mechanisms are alterations in metabolism of insulin and insulin-like growth factors (IGFs), altered levels of adipocytokines and immune function. Whether the same

mechanisms apply to BRCA-associated breast cancer is yet unknown. The risk-reducing effects prophylactic (salpingo-)oophorectomy in BRCA1/2 carriers[72] suggests that hormonal influences are important, despite the fact that BRCA1 breast cancers have a predominantly negative estrogen receptor status[73]. In other words, estrogen may play a role in the early steps of BRCA1-related carcinogenesis[74]. BRCA1 may function as part of a feedback mechanism to regulate estrogen signalling by functioning as a brake on ERα-driven proliferation and that BRCA1 mutations released this brake[75].
This would also explain the organ specificity of carcinogenesis. Foulkes proposed that the clinical, molecular, and pathologic features of BRCA1-associated breast cancer suggest the possibility that BRCA1 may function as a stem-cell regulator[76]. In vitro and mouse models for breast stem-cell function showed that loss of BRCA1 expression may result in an accumulation of genetically unstable breast stem cells, providing targets for further carcinogenic events[77]. So far, the association between hormonal risk factors, like parity and use of oral contraceptives, and BRCA-related breast cancer seems to be similar to what is observed in the general population[78-80]. However, in the general population, the effect of body weight on breast cancer risk is stronger for estrogen receptor positive tumours than for estrogen negative tumours[81]. Unfortunately, for the studies described in this thesis, information on estrogen and progesterone receptor status was not available, however, information on tumour characteristics will be collected as part of the HEBON Research Facility. Incorporating information on receptor status in the body weight study as well as in studies on, for example, the breast cancer risk reducing effect of prophylactic (salpingo-) oophorectomy would be valuable.
In the general population, estrogen action is strongly related to the IGF system[82] and IGF-1 has been found to be positively associated with breast cancer, with stronger associations observed for premenopausal breast cancer than for postmenopausal breast cancer[83,84]. Interestingly, a recent study identified statistically significant associations in variants in IGF1R and IRS1 in BRCA1 carriers and IGFBP2 in BRCA2 carriers[85]. However, another study observed no modifying effect of the IGF-1 gene on breast cancer risk in BRCA1/2 mutation carriers[86].
In vivo, increased levels of physical activity have been associated with decreased levels of serum estrogens[87]. As studies on risk factors and BRCA-associated breast cancer show that the associations, including our physical activity and body weight studies, seem to be similar to what is known in general population, we expect that a similar effect of exercise on serum estrogen levels may be observed in BRCA1/2 mutation carriers. To our knowledge, no such studies have been conducted so far. There is an advanced preclinical mouse model for BRCA1-associated breast cancer[88] which would be suitable for conducting a study on, for example, the effects of exercise and/or diet and/or weight loss on hormone levels. Another approach or next step may be an intervention study in BRCA1/2 mutation carriers on the effect of a combination of dietary weight loss and physical activity on endogenous hormone levels (and breast density). However, such a study trial may not be feasible considering for example the relatively high uptake of prophylactic surgery.

ENERGY BALANCE

Energy balance is the balance between energy intake and energy expenditure. In the general population, high levels of energy intake have not been consistently related with breast cancer, perhaps because women that are highly active may eat more calories. Few studies have investigated the joint effects of the individual components of energy balance on the risk of breast cancer but they all confirmed the hypothesis that women with the most unfavourable energy balance profile (i.e. women that are least active, have the highest energy intake, and with higher levels of adiposity) have the highest risk of developing breast cancer[89].

To our knowledge, only two small studies have examined the association between energy intake and breast cancer risk in BRCA1/2 mutation carriers. The first was a case-control study by Nkondjock et al. among 137 BRCA carriers[90]. They observed a positive association between total energy intake and BRCA-associated breast cancer risk. This effect was independent of age, maximum lifetime BMI and physical activity, however, the analyses were not adjusted for menopausal status. The second study, conducted by the same group of investigators, had a case-only design and included 738 breast cancer patients comprising 38 BRCA mutation carriers[91]. Total energy intake was not statistically significantly different between the study groups (i.e. BRCA carriers and BRCA non-carriers). However, the study sample was small, the analyses were not adjusted for physical activity, and it was not clear whether BMI was adjusted for. Additionally, the food frequency questionnaire that was used in both studies covered only a 1-year period prior to the breast cancer diagnosis and a corresponding period for the controls. Clearly, more data is needed on the association between energy balance and breast cancer risk in BRCA1/2 mutation carriers. Future research should focus on the joint effects of body weight, physical activity, and energy intake and possible determinants of weight change in BRCA1/2 mutation carriers. As these are some of the few more modifiable risk factors, they may provide a target to add to breast cancer prevention in this high-risk population.

CONCLUSIONS

The studies described in this thesis add support and complementary data to other epidemiological evidence on the association of breast cancer risk with diagnostic radiation, physical activity, and body weight in BRCA1/2 mutation carriers. We showed that exposure to diagnostic radiation before age 30 was associated with an increased breast cancer risk in BRCA1/2 mutation carriers, at dose levels considerably lower than at which increases have been found in other radiation-exposed cohorts. Two methodological studies confirmed that the extent of the observed misclassification of self-reported diagnostic radiation history by BRCA1/2 mutation carriers was small and largely non-differential by case-status. Our study on the association between physical activity and breast cancer risk showed that BRCA1/2 mutation carriers may reduce their risk of breast cancer by participating in sports activity, preferably from early ages onwards. The study on the association between body weight and BRCA-related breast

cancer risk showed that BRCA1/2 mutation carriers may reduce their breast cancer risk by maintaining a healthy body weight throughout life, but the association was only observed for postmenopausal breast cancer. These results require confirmation by future studies focussing on prospective follow-up.

(CLINICAL) IMPLICATIONS

IMPLICATIONS BASED ON THIS THESIS

The results described in this thesis may have several implications for daily clinical practice and the counseling of BRCA1/2 mutation carriers.

With respect to the diagnostic radiation study, we recommend that physicians follow the existing breast cancer screening guidelines[92-94], so avoiding mammographic screening before age 30 and using magnetic resonance imaging (MRI) as the main tool for surveillance at young ages in BRCA1/2 mutation carriers. Additionally, increasing the age at which mammographic screening should start from 30 to 35 or 40 years may be considered. In general, adaptation of the current screening protocol should preferably be based on new data obtained from a randomised trial comparing, for example, MRI and mammography versus MRI alone for screening at ages[30-40], stratified by low versus high breast density assessed on age 30. However, such a trial may not be feasible considering the relatively high uptake of prophylactic surgery. As a result of having a 50% chance of being a carrier, untested daughters of carriers are potentially increased radiosensitive as well. In an era of increasing collective dose from medical radiation, mostly due to increased use of CT scans, the medical need for diagnostic procedures using ionizing radiation must be balanced against the potential radiation risk, especially during childhood and adolescence. Referring physicians and radiologists should continue to consider possibilities to reduce exposure as low as reasonably achievable (ALARA) by using settings customized for children and performing only necessary procedures.

We do not advocate genetic testing in children as research shows that genetic testing of children has profound psychological effects and is often not to the advantage of the child at risk[95]. However, BRCA1/2 mutation carriers should be informed about the potential risk of early exposure to ionizing radiation as women may be less likely to ask for a diagnostic procedure to be performed on their children in case of a suspected not life-threatening condition. On the other hand, we must ensure that informing these women will not cause anxiety around screening and necessary diagnostic procedures.

The diagnostic radiation study showed statistically significant associations at dose levels considerably lower than at which increases have been found in other radiation-exposed cohorts. Studies on other risk factors, including our physical activity and body weight studies, show that the associations observed in BRCA1/2 mutation carriers seem to be similar to what is known in general population. Despite the requirement of confirmation in prospective studies, these studies confirm that risk avoidance behaviour (i.e. a healthy lifestyle and an advice on the use of exogenous hormones, like oral contraceptives and hormone replacement therapy) may be part of genetic counseling already. BRCA1/2 mutation carriers should be informed about the associations between risk factors and

breast cancer risk as they exist in the general population. In carriers, risk factors may have a larger effect in absolute terms due to the high background risk, even if their relative risk is the same. However, the communication on risk avoidance behaviour should be evidence- and consensus-based, thus a protocol should be developed and discussed within the HEBON and other international gremia before this can be implemented. Furthermore, it should be emphasized to women that lifestyle changes can not replace other risk reducing strategies and is not as effective as RRM and/or BPSO.

WEIGHING OPTIONS FOR CANCER RISK REDUCTION IN BRCA1/2 MUTATION CARRIERS

BRCA1/2 mutation carriers weighing their options for cancer risk reduction strategies make very different choices about how to manage their cancer risks, depending on their age, family history, reproductive history, concurrent diagnoses, and personal preferences. Currently, the risk reducing strategies for carriers are prophylactic surgery and breast screening incorporating MRI for early detection. Many carriers choose to undergo prophylactic surgery despite its disadvantages (e.g. physical effects and impact on quality of life). The process of decision-making is complex and uptake of prophylactic surgery mainly depends upon the magnitude of risk, age, and timing in life. A reduction in the proportion of carriers undergoing prophylactic surgery in the future may be expected for several reasons. First, recent breast cancer risk estimates observed in population-based studies are somewhat lower than the early family-based studies. Second, optimization of BRCA mutation-specific penetrance estimates will result in lower risks for part of the carriers who now opt for prophylactic screening based on average risk estimates. Third, overestimation of risk is a common finding in women with a family history of breast cancer attending genetic counseling so appropriate risk prediction modelling and risk communication may provide women with perceived risks closer to actual risk. On the other hand, several studies have indicated that the penetrance of BRCA1 and BRCA2 mutations has increased in recent generations. Moreover, several studies show that women often decide a priori to undergo prophylactic surgery if the test result would indicate that they are a mutation carrier, and few of them change this decision afterwards[96,97]. An increasing problem is that women may retrieve information on risk reducing strategies on the World Wide Web, which may not necessarily originate from a reliable source. At present, it is unknown which lifetime risk outweighs the option of prophylactic surgery and it is likely that this risk is not the same for every woman.
Over the past decade, breast screening protocols for BRCA1/2 mutation carriers have been improved to a level where the combination of mammography and MRI detects twice as many cancers as does simultaneously performed mammography. This has resulted in interval cancer rates below 10%. While a reduction in mortality has not yet been demonstrated, when used in conjunction with mammography, MRI appears to result in early breast cancer detection in BRCA-mutation carriers; with 75% to 94% of screen-detected invasive tumours being 2 cm or smaller at diagnosis and 75% to 83% being negative for axillary nodal metastases. The increased sensitivity of MRI was initially undermined by a lack of specificity. With growing experience, the specificity

of MRI detection, including detection of DCIS, has improved to a similar level as achieved with mammography. Still, many other improvements can be made as well. The current recommendation is performing yearly MRI screening starting at age 25 and yearly mammography from age 30 onwards. MRI screening is time consuming and relatively expensive but readily available for screening BRCA1/2 mutation carriers in the Netherlands. However, in other countries, this may not (yet) be the case. The application of digital mammography may reduce the radiation dose around 50%. However, the development of digital mammography has not prevented an increase in average glandular dose for mammography, which is likely caused by changes in screen-film products and processing techniques[98]. For BRCA1/2 mutation carriers there should be active prevention to reduce or eliminate this so-called "dose-creeping".
An interesting option to add to the existing breast cancer screening protocol in the near future is breast density-based screening. In the general population, breast density is a strong risk factor for breast cancer. If a similar association exists in BRCA1/2 mutation carriers, as the few published studies conducted seem to indicate, carriers with a relatively high breast density might benefit from a higher frequency of screening. Furthermore, a high breast density hampers diagnosis by mammography; so mammographic screening of carriers with dense breast tissue from age 30 onwards may not be effective. In general, as previously mentioned, adaptation of the current screening protocol should preferably be based on new data obtained from a randomised trial.
Ultimately, each woman must make her own personal decision about the best strategy for managing her breast cancer risk conveyed by a BRCA1 or BRC2 mutation. However, physicians need to ensure that women make these decisions after being well-informed about the actual risk and potential modifiers. Additionally, risk communication may require improvement, to achieve that perceived risks become closer to actual risks. Existing risk prediction models, like the Gail model, for breast cancer have limited predictive value, probably because they are either based on family history alone, or on hormonal/life style factors alone. Detailed knowledge of genetic susceptibility and interactions with hormonal/lifestyle factors will result in much more accurate individual risk prediction and improved genetic counseling for women at high familial risk of breast and ovarian cancer.
An important related question is whether BRCA1/2 mutation carriers are interested in changing their lifestyle behaviour and willing to change them. Furthermore, the fact of knowing the possible reduction of the risk associated with adopting a behaviour and the willingness to change a behaviour may be insufficient to generate behavioural changes. Moreover, women who opt for BRCA1/2 genetic testing perhaps already have a relatively healthy lifestyle. As already indicated earlier in this chapter, there is hardly any literature on testing behaviour based on risk factors for breast cancer. To our knowledge, only three relatively small studies investigated short term changes in lifestyle factors following BRCA1/2 genetic testing[99-101]. Two studies observed no change in diet and physical activity among 46 and 201 BRCA1/2 mutation carriers within 6 months after genetic testing and counseling process[99,100]. Rouleau et al. observed a trend toward decreased HRT use among BRCA1/2 mutation from the first genetic counseling session

up to 1 year post-disclosure of BRCA1/2 test results[101]. In the latter study it was unclear how many of the 31 ever HRT-users were carrier of a BRCA1/2 mutation. Interestingly, a recent qualitative study among 22 adult offspring who learned of their parent's BRCA mutation prior to age 25 suggested that, among the offspring, many tobacco users reported to have stopped smoking[102]. However, in the offspring reports, cancer risks and offspring genetic testing were described more frequently than risk modification strategies.

RECOMMENDATIONS FOR FUTURE RESEARCH

RECOMMENDATIONS BASED ON THIS THESIS

In general, our findings require confirmation by future studies focusing on prospective follow-up in larger sample sizes.

Future studies should also focus on the investigation of potential testing bias based on reproductive characteristics and lifestyle factors. Because the HEBON study includes both tested and untested individuals, it provides a unique source of information that can be used to study testing behaviour although results are likely to be cultural-dependent. Furthermore, as long as prospective studies lack power, larger studies are needed to confirm the small underreporting of age at first mammography by cases and the more variable pattern among unaffected carriers. However, validation of self-reported exposure requires a gold standard which, unfortunately, does not exist for all potential exposures of interest. Additionally, knowing which cues are used by participants to retrieve information on exposure in the past will help to improve measurement instruments. Survival bias was an important feature of studies described in this thesis.

Current studies on prognostic and predictive factors in BRCA-associated breast cancer focus on tumour characteristics and treatment. However, for example, in the general population both physical activity and body weight have an effect on prognosis, so a potential prognostic effect in BRCA-associated breast cancer can not be excluded and warrants further investigation.

With respect to the diagnostic radiation study, investigation of the potential association between occupational exposure to ionizing radiation and breast cancer risk in BRCA1/2 carriers is important as an increased risk may have major implications for the choice of occupation in BRCA1/2 carriers. Additionally, future studies should also address modifying effects by genotype, for both low and high dose ionizing radiation exposure.

With respect to the physical activity and body weight studies, future research should focus on the joint effects of body weight, physical activity, and energy intake and possible determinants of weight change in BRCA1/2 mutation carriers. Studies should specifically focus on the investigation of different types and dimensions (intensity, frequency and duration) of physical activity and whether physical activity in specific time periods is most effective for lowering breast cancer risk. Future studies investigating body weight and BRCA-associated breast cancer risk should at all times be stratified according to menopausal status. Investigation of the effects of modifiers of BRCA-associated breast cancer risk that may act through hormonal-related pathways, like

physical activity, body weight, and BPSO, on hormone levels in BRCA1/2 mutation carriers are required to elucidate potential biological mechanisms. Another approach that may add to the knowledge about the development of BRCA-tumours is by stratifying the results by hormone receptor status.

GENERAL RECOMMENDATIONS

The multidisciplinary field of BRCA-associated breast cancer research is evolving quickly. New data from unbiased, preferably prospective, studies are required to confirm the results of the studies described in this thesis. As carriers of BRCA1 and BRCA2 mutations are relatively rare and incident case numbers are not expected to increase rapidly due to the relatively high uptake of prophylactic surgery, these studies should be conducted within the framework of international multicentre collaborations and/or in countries where the uptake of prophylactic surgery is still low, like in Israel and Korea. These large international studies are also required to investigate BRCA mutation-specific penetrance to optimize risk estimates and the impact of modifying genes and other hormonal and lifestyle factors. Such an initiative has recently been established: the Breast Cancer Association Consortium (BCAC) and The Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) were founded in 2004 and 2005, respectively. Presently, the BCAC includes all major research groups in Europe, USA, Asia and Australia and has amassed genotype and phenotype data on over 58,000 cases and 64,000 controls. Likewise, CIMBA has collected similar data on almost 22,000 BRCA1/2 mutation carriers. A well-designed, up-to-date risk prediction tool including a risk management decision tool should be developed to improve individual age-specific risk communication and to facilitate decision making in BRCA1/2 mutation carriers. This tool should use data on BRCA mutation-specific penetrance estimates and the impact of modifying genes and other risk factors, like reproductive factors, use of oral contraceptives, lifestyle behaviours, and radiation exposure. Furthermore, this tool should be able to assess both breast and ovarian cancer risk combined as risk factors may affect risks of these cancers differently (e.g. oral contraceptives). For women who already developed breast cancer, information on tumour characteristics (histopathological and genetic profile of the tumour), treatment, and lifestyle factors as prognostic factors (e.g. body weight after diagnosis) should be incorporated. Moreover, the tool should provide evidence-based information on cancer incidence or recurrence, overall survival and breast and ovarian cancer specific survival. More research is needed to assess whether early detection of BRCA-associated breast cancer by the current screening guidelines and risk reducing mastectomy reduce mortality. Additionally, studies should determine the size of benefits judged as sufficient to make prophylactic surgery or lifestyle changes worthwhile.

To date, the majority of studies assessing the influence of hormonal and lifestyle risk factors on breast cancer risk in BRCA1/2 mutation carriers are carrier-only studies while comparison with non-carriers is necessary to assess whether there is gene-risk factor interaction (i.e. whether the effects of risk factors are different in carriers versus non-carriers). Future research should therefore also focus on assessing actual gene-risk factor interactions. The assessment of gene-risk factor interactions in epidemiological

studies may help identify potential biological pathways. Because the HEBON study includes both carriers and non-carriers, it provides a unique source of information that can be used to study gene-risk factor interactions.
As a result of consortia like BCAC and CIMBA, knowledge on intermediate and low risk breast cancer genes is growing, although this information is not yet applied into clinical practice. Future research should also focus on the group of women carrying these intermediate and low risk genes and potential interactions with hormonal/lifestyle risk factors.

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