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GENERAL INTRODUCTION

INTRODUCTION

Breast cancer is the most common malignancy among women in developed countries[1]. In the Netherlands, each year, approximately 12,000 women are diagnosed with breast cancer[2]. Among women with breast cancer, 13% report to have at least one first-degree relative with a history of breast cancer, while among controls 7% report to have a positive family history[3]. In 1994 and 1995, two genes associated with a strong increased risk of developing breast and/or ovarian cancer were identified: BRCA1 on chromosome 17q21[4] and BRCA2 on chromosome 13q12-13[5]. BRCA1 and BRCA2 are transmitted in an autosomal dominant pattern and are both tumour suppressor genes that play a role in the repair of DNA damage[6]. Together with other high-risk breast cancer susceptibility genes, like TP53 and PTEN, they account for less than 30% of the familial component of breast cancer[7]. The residual genetic variance is due to moderate- and low-risk breast cancer susceptibility alleles (e.g. CHEK2[8], CASP8[9] and FGFR2[10]) and as yet unidentified genes (see Figure 1).

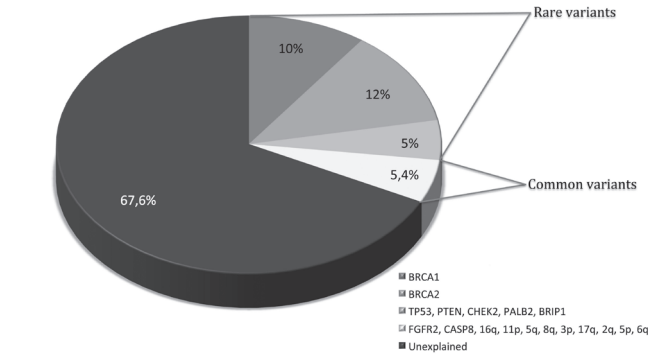

FIGURE 1. Fraction of the excess familial risk of breast cancer explained by the currently known susceptibility genes/loci. This assumes that most of the familial risk is due to shared genes rather than shared environment.[11]

RISK OF BREAST AND OTHER CANCERS IN BRCA1/2 MUTATION CARRIERS

According to estimates of lifetime risk, in the Netherlands, about 11% of women in the general population will develop breast cancer[12]. The penetrance estimates for women with an altered BRCA1 or BRCA2 gene vary between 40 and 80%[13-19]. Family-based studies[13-15] observed higher cumulative risks (68-85% for BRCA1 and 75-84% for BRCA2) than population-based studies (45-65% for BRCA1 and 40-45% for BRCA2)[16-19]. Chen et al. reported meta-analytic mean cumulative breast cancer risks at age 70 of 57% (95% confidence interval (CI), 47% to 66%) for BRCA1 and 49% (95% CI, 40% to 57%) for BRCA2 mutation carriers (see Figure 2)[20].

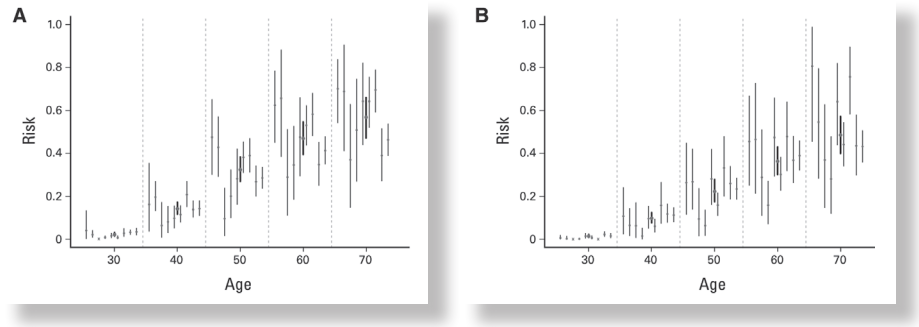

FIGURE 2. The cumulative breast cancer risk estimates from published studies (thin vertical bars) and the meta-analytic mean (thick vertical bars, height represents 95% CIs) for (A) BRCA1 carriers and (B) BRCA2 carriers[20].

The lifetime risk of ovarian cancer varies between 30 and 60% for BRCA1 and 5-20% for BRCA2 mutation carriers[13-20]. In addition, BRCA1/2 gene mutations may increase the risk of other cancer sites. BRCA1 mutations have been associated with an increased risk of pancreatic cancer and cancer of the uterine body, cervix cancer, and prostate cancer[21]. In BRCA2 mutation carriers, increased risks of prostate cancer, melanoma, and cancers of the pancreas, gallbladder, bile duct, and stomach have been reported[22,23]. Male BRCA1/2 mutation carriers also have an increased risk of breast cancer, primarily if the alteration is in BRCA2[24,25].

CLINICAL FEATURES OF BRCA-ASSOCIATED BREAST CANCER

BRCA-associated breast cancer is characterized by a relatively early age at onset[20]. BRCA1 and BRCA2 mutations predispose to different age-specific risks of breast cancer. In pooled pedigree data from 22 studies, the risk of breast cancer in BRCA1 carriers, relative to the general population, declined with age from over 30-fold at <40 years of age to 14-fold at >60 years of age[16]. By contrast, the relative risk in BRCA2 carriers was approximately 11-fold increased in all age groups >40 years, and not significantly higher at earlier ages[16]. The risk of contralateral breast cancer has been found to be significantly increased in BRCA1/2 mutation carriers compared with controls with an estimated 10-year risk ranging from 20 to 42% versus 5 to 6%, respectively[26].
The frequency of BRCA mutations in unselected bilateral breast cancer cases is approximately 5%, although this may exceed 20% in early-onset and/or familial forms of the disease[27].
BRCA1 and BRCA2 mutations predispose to different subtypes of breast cancer. BRCA1-associated breast tumours cluster in a basal-like gene expression pattern, or subtype[28]. Only approximately 10% of BRCA1 tumours show any positive staining for the estrogen receptor (ER), compared to 65% of sporadic cases[29]. BRCA1 tumours are also often progesterone receptor negative (79%) and Her2-neu negative (97%)[29]. BRCA2-associated breast tumours tend to be ER and PR positive and Her2-neu negative, which is similar to sporadic cases[29].

So far, studies have observed, for the most part, no overall survival difference between BRCA-associated breast cancers and sporadic breast cancers[26].

RISK REDUCING STRATEGIES

Several approaches are available for managing breast cancer risk in BRCA1/2 mutation carriers. The possible risk reducing strategies are:

- screening for early detection
- prophylactic surgery (breasts and/or ovaries)
- chemoprevention
- other preventive approaches: risk avoidance behaviour (i.e. hormonal and lifestyle factors)

SCREENING

If breast cancer develops, it is important to detect it at an early stage. In the Netherlands, the current screening guideline[30] for BRCA1/2 mutation carriers (and those who have a 50% chance of being a carrier) recommends:

- At ages 25-60 yearly magnetic resonance imaging (MRI)
- At ages 30-60 yearly mammography
- At ages 25-60 yearly clinical breast examination
- At ages 60-75 participation in population-based screening programme
- After prophylactic mastectomy there is no indication for screening
- Screening is to be performed by clinical genetic centre with a multidisciplinary team

In the past (i.e. before BRCA discovery), clinicians were already aware that there were families that showed an autosomal dominant pattern of transmission of hereditary breast cancer, which led to screening outside the population-based screening program based on positive family history. Screening often started at a young age, 25-35 years. The initial guidelines included a 6-monthly clinical breast examination, annual mammography and instructions for monthly breast self-examination.
In the 1990s, breast screening studies including MRI were initiated in high-risk women because it was proven that MRI could detect breast cancers that were occult at mammography and clinical examination[31]. MRI appeared to be a more sensitive screening method than mammography, also in young women with dense breast tissue, which hampers diagnosis by mammography[32]. The increased sensitivity of MRI was initially undermined by a lack of specificity. With growing experience, the specificity of MRI detection has improved to a similar level as achieved with mammography[33,34]. For the detection of ductal carcinoma in situ (DCIS) there exists a learning curve as well[33,34]. Despite promising results in one single-centre publication[35], mammography still shows a higher sensitivity than MRI for the detection of these pre-invasive lesions[36].
In the general population, population-based breast cancer screening by mammography in women 50-75 years of age reduces mortality and is cost-effective[37,38]. Whether early detection of BRCA-associated breast cancer leads to a better prognosis is yet unknown, although current screening recommendations assume that this is the case.

PROPHYLACTIC SURGERY

Prophylactic surgery is currently the only option that leads to a demonstrable reduction in breast cancer risk in BRCA1/2 mutation carriers and includes (bilateral) risk reducing mastectomy (RRM) and bilateral prophylactic (salpingo-) oophorectomy (BPSO). RRM does not eliminate the risk of breast cancer completely because depending on the type of mastectomy, there is always a certain amount of breast tissue remaining in the surgical area. In women with no previous history of breast cancer, RRM reduces the risk of breast cancer between 91 and 100% in 3- to 7-year follow-up[39]. In the Netherlands, two studies on the risk of primary breast cancer after RRM have been conducted[40,41]. Both originated from the Rotterdam Family Cancer Clinic, one of the specialised centres. No cases of primary breast cancer occurred after RRM in 3[40] and 4.5 years[41] of follow-up. Prophylactic contralateral mastectomy after unilateral breast cancer diagnosis reduces the risk of contralateral breast cancer by 91 to 97%. BPSO reduces the risk of breast cancer by 50% in carriers with no previous history of breast cancer[42] indicating that BRCA-associated carcinogenesis is hormone-sensitive despite the predominantly negative ER status of BRCA1 breast cancers[43,44].
The uptake of RRM and BPSO varies enormously worldwide. For example, 36% of U.S. BRCA1/2 mutation carriers undergo RRM while in Polish carriers this rate is only 3%[45]. However, the overall uptake after long term follow-up is likely to be higher than these previous estimates[46]. At the Netherlands Cancer Institute, the impression is that the current estimates of cumulative uptake rates at age 45 is about 50% for RRM and that a large majority of BRCA1 and BRCA2 carriers opt for BPSO (dr. S. Verhoef, clinical geneticist, Family Cancer Clinic, Netherlands Cancer institute, Amsterdam, the Netherlands, *personal communication*).
Earlier published uptake rates from the Rotterdam Family Cancer Clinic were 35-51% for RRM and 49-64% for BPSO in unaffected carriers[47,48]. In the Netherlands, BPSO is currently recommended to proven BRCA1/2 mutation carriers from age 40 onwards (dr. S. Verhoef, *personal communication*). BPSO in BRCA1 carriers may also be performed before age 40 when childbearing is complete. Uptake of RRM is optional; there are no specific recommendations but clinical geneticists provide information on the current evidence on the efficacy and disadvantages. RRM almost eliminates the incidence of breast cancer and thereby prevents diagnosis and treatment; however, there is no evidence that RRM reduces mortality. Even though RRM and/or BPSO may be acceptable options, these procedures have a number of disadvantages. Premenopausal BPSO induces menopause, with loss of fertility, potential problems with sexual functioning, and increased risks of osteoporosis and cardiovascular diseases[49]. Disadvantages of RRM include post-operative surgical complications, unanticipated re-operations, and physical morbidity[50]. Most studies on psychosocial measures report decreased cancer-related stress after RRM and high levels of satisfaction with the decision to have RRM but more variable satisfaction with the cosmetic result[50].

CHEMOPREVENTION

The use of natural or synthetic substances to reduce the risk of breast cancer in BRCA1/2 mutation carriers is an interesting potential strategy. However, few studies have been performed to test the effectiveness of such agents in BRCA1/2 mutation carriers. In the Breast Cancer Prevention Trial, small subgroup analyses showed that tamoxifen was associated with a statistically non-significantly increased risk in BRCA1 mutation carriers, while in BRCA2 mutation carriers a non-significantly reduced risk was observed[51]. A reduced risk of contralateral breast cancer among BRCA1 and BRCA2 breast cancer patients who used tamoxifen was found[52,53]. Trials with tamoxifen and other agents, like aromatase inhibitors, in BRCA1/2 mutation carriers are anticipated. Chemoprevention as a choice for risk management of breast cancer in BRCA1/2 mutation carriers is currently not available in the Netherlands.

OTHER PREVENTIVE APPROACHES: RISK AVOIDANCE BEHAVIOUR

Theoretically, breast cancer risk in BRCA1/2 mutation carriers could also be modified by interventions in hormonal and lifestyle risk factors, like use of oral contraceptives and physical activity, as for many of these risk factors the association with breast cancer risk in the general population is established. However, few studies have investigated these associations in BRCA1/2 mutation carriers and often showed inconsistent results. Currently, risk avoidance behaviour is not part of genetic counseling.

VARIATION IN BRCA-ASSOCIATED BREAST CANCER RISK

Studies have shown that penetrance, age at onset, and phenotypic expression vary substantially between and within BRCA1/2 families (see Figure 2), suggesting that the development of breast cancer may also be influenced by the specific mutation, modifier genes and non-genetic risk factors. For example, it has been proposed that the penetrance of Ashkenazi BRCA2 founder mutation 6174delT is less than that of other BRCA2 mutations[54,55]. RAD51 is the first gene to be reliably identified as a modifier of risk among BRCA2 mutation carriers; breast cancer risk was non-significantly increased 1.2-fold among heterozygotes, while among homozygotes, breast cancer risk was increased over 3-fold (heterogeneity test $P<0.001$)[56]. Furthermore, the risks are expected to be affected by family history due to shared genes and/or environment. Few studies have investigated whether family history of breast cancer is a modifier of breast cancer risk in BRCA1/2 mutation carriers and show small non-significant associations[57-59]. Recently, some of the common susceptibility alleles that are associated with breast cancer risk in the general population[10], were found to affect breast cancer risk in BRCA1/2 mutation carriers[60]. Additionally, 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[16,18,61].

The focus of this thesis is on the association between non-genetic modifiers of breast cancer risk in female BRCA1/2 mutation carriers.

NON-GENETIC MODIFIERS OF BREAST CANCER RISK IN BRCA1/2 MUTATION CARRIERS

Established non-genetic risk factors for breast cancer in the general population and associated relative risks are listed in Table 1. For many of these factors, it is not yet known whether they influence breast cancer risk in BRCA1/2 mutation carriers and what the magnitude of a potential risk increase or decrease is. The majority of studies on potential modification of breast cancer risk in BRCA1/2 mutation carriers by non-genetic risk factors have so far focused on reproductive and exogenous hormonal factors. Some of the associations between these factors and BRCA-associated breast cancer have been found to be similar to what is observed in the general population. A younger age at menarche and a later age at menopause increase the risk of breast cancer in BRCA1/2 mutation carriers[62,63]. Multiparity appears to be protective in BRCA1/2 mutation carriers[64-67]. Some studies indicate that breastfeeding and a younger age at first full term pregnancy may reduce the risk of breast cancer in carriers as well, but others did not observe such an association[64,67-70]. The use of exogenous hormones is currently a controversial topic. Although oral contraceptives have repeatedly been proposed as a potential chemoprevention strategy for ovarian cancer in BRCA1/2 mutation carriers[71,72], its use potentially increases the risk of breast cancer[73,74]. In the case of a hereditary predisposition this may have a larger effect in absolute terms due to the high background risk, even if the relative risk is the same. However, for the moment the evidence is deemed too scarce to actively discourage use of oral

TABLE 1. Established risk factors for breast cancer in the general population

Risk factor	Categories	Relative risk
Age at menarche	< 12 versus > 12 years	1.2
Age at menopause	> 54 versus < 45 years	2.0
Endogenous hormones	Q4 versus Q1	2.0-2.5
Mammographic density	> 75% versus < 25%	3.0-4.0
Personal history of breast cancer	Yes versus no	2.0-5.0
Number of first degree family members with breast cancer	1 versus 0	1.8
	2 versus 0	2.9
	3 versus 0	3.9
Number of children	Per child	0.93
Duration of breastfeeding	Per 12 months	0.95
Age at first full term pregnancy	Per year younger	0.97
Use of oral contraceptives	Current use versus no use	1.24
Use of HRT	Current use (> 5 years) versus no use	1.35
Use of DES	Yes versus no	1.3
Alcohol consumption	Per glass per day	1.08
Ionizing radiation[82,83]	> 40 Gy versus < 4 Gy	8.0-11.0
Physical activity	Per hour per week	0.92-0.97
Overweight; postmenopausal breast cancer	BMI > 25 versus BMI 20-24 kg/m^2	1.3-1.4
Weight gain; postmenopausal breast cancer	Per 5 kg gain	1.08
Height	> 1.70 m versus < 1.70 m	1.20-1.25

Modified with permission from chapter 2 of Wobbes Th., Nortier J.W.R. & Koning C.C.E. Mammacarcinoom 2007; De Tijdstroom, Utrecht[84]

contraceptives in BRCA1/2 mutation carriers. No significant association was observed between ever use of hormonal replacement therapy (HRT) and risk of breast cancer among BRCA1/2 mutation carriers, however, the authors observed that HRT use starting soon after menopause increased breast cancer risk in BRCA1/2 mutation carriers (Brohet et al., *submitted*), which was similar to what is observed in the general population. The Hereditary Breast Cancer Clinical Study Group investigated the use of infertility medications and abortions and observed no association with breast cancer risk in BRCA1/2 mutation carriers[75,76]. A number of studies conducted on the potential association between dietary habits (energy intake, coffee consumption and alcohol consumption) and smoking with breast cancer risk in carriers is limited and the results were inconsistent[77-81].
The focus of this thesis is on the association between diagnostic radiation, physical activity, and body weight with breast cancer risk in female BRCA1/2 mutation carriers. The few studies that have been conducted on these topics so far are described in more detail below, as well as potential biological mechanisms.

DIAGNOSTIC RADIATION

Exposure to ionizing radiation is an established risk factor for breast cancer in the general population[85]. The risk appears to decrease with increasing age at exposure[86]. The association follows a linear non-threshold model which is widely accepted to apply to risk estimation, and used in radiation protection[87,88]. In the general population, a minimal induction time for breast cancer after exposure to radiation of 10 to 15 years is generally accepted[86]. The evidence for an increased breast cancer risk after high doses of ionizing radiation (e.g. survivors of childhood and adolescent cancer who had radiotherapy involving the chest; 4-40 Gy[82,83]) and intermediate doses (e.g. the Life Span Study of Japanese atomic bomb survivors; 0-6 Sv[89] and the study on irradiation of the thymus in infancy; 0.71 Gy[90]) has long been established. However, questions remain about the existence and possible magnitude of risk following low doses (i.e. <0.1 Gy) such as those used in diagnostic procedures[91]. Examples of diagnostic procedures are fluoroscopies, X-rays, mammograms, and CT-scans. The estimated breast doses of such diagnostic procedures range from 0.0005 to 0.02 Gy (see Table 2, chapter 2). Ronckers et al. reported a borderline-significant radiation dose response association between X-rays at young ages for abnormal spinal curvature based on 78 cases of invasive breast cancer in 35.5 years of follow-up (mean dose 0.011 Gy; excess relative risk per Gy = 2.86, p=0.058)[92]. Other diagnostic radiation studies observed increased breast cancer risks as well but the mean doses in these studies exceeded 0.1 Gy[93-95].
Ionizing radiation causes DNA double strand breaks, which are repaired in normal cells, in part, by homologous recombination based mechanisms. BRCA1/2 mutation carriers might be more susceptible to radiation-induced breast cancer than non-carriers, or the general population. Functional BRCA1 and BRCA2 proteins are required for efficient repair by homologous recombination and genomic stability[6]. In the absence of BRCA1 or BRCA2 proteins the repair of DNA damage, caused by ionizing radiation, is impaired[96-98] because alternative repair pathways, such as non homologous end joining and single

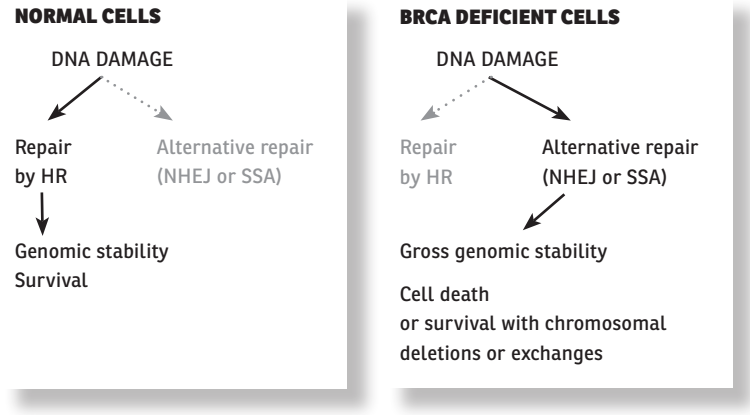

FIGURE 3. Loss of functional BRCA1 or BRCA2 affects the choice of DNA double-strand break repair pathway[99] (HR, homologous recombination; NHEJ, non homologous end joining; SSA, single strand DNA annealing)

strand DNA annealing, are utilized leading to cell death or survival with genomic damage providing targets for further carcinogenic events (Figure 3).
Because BRCA1/2 mutation carriers are frequently screened by mammography from a relatively young age onwards, the potential hazardous effect of mammographic screening at young ages is a major concern and should be balanced against its potential benefit (i.e. early detection of breast cancer with better prognosis). Already in 1989, before the BRCA genes were identified[4,5], concerns were expressed that genetically predisposed women might have an increased risk of radiation-induced breast cancer[100,101]. More recently, two studies[102,103] that investigated the association between routine chest X-rays and breast cancer risk of carriers found that any reported exposure was associated with an increased risk in breast cancer. Elevated risks were observed especially for those exposed before age 20 only, and at dose levels considerably lower than at which increases have been found in other radiation-exposed cohorts. The IBCCS X-ray study also observed that risk was particularly increased for women aged 40 years and younger and for women born after 1949[102]. Two other carrier studies[104,105] focussing on mammography exposure and breast cancer risk, did not observe an association. Two important limitations of these four studies was the investigation of a single low dose ionizing radiation source (i.e. only chest X-rays or only mammograms) and potential recall bias (i.e. differential misclassification of self-reported exposure based on case-status).
The lifetime risk of developing radiation-induced breast cancer is age-dependent. For a women from the general population aged 50-65 years, the risk is estimated to be 1 per million per mGy dose, and for a women aged 25-30, this risk is doubled[106,107]. In the Dutch population-based screening program the ratio of the number of screen-detected to radiation-induced lethal tumours is estimated at 250-400:1[108]; this ratio deteriorates with increasing breast density[106]. It is plausible that the ratio of screen-detected to radiation-induced tumours is more unfavourable for BRCA1/2 mutation carriers than for women from the general population. Recently, Berrington de Gonzalez et al.

estimated the reduction in breast cancer mortality required to outweigh the radiation risk in BRCA1/2 mutation carriers[109]. Under the assumption that the mortality reduction from mammography is 15%-25% or less for young women, they concluded that there would be no net benefit from annual mammographic screening of BRCA mutation carriers at age 25-29 years; the net benefit would be zero or small at age 30-34 years, but there should be some net benefit at age 35 or older. However, this was based on a multiplicative risk model; assuming an interaction effect of 1.5 (based on only one study[110]) then the ratio of breast cancer mortality from screening required to outweigh the radiation risk would be increased by 1.5.

PHYSICAL ACTIVITY

In the general population, it has been established that increased levels of physical activity decrease the risk of breast cancer by approximately 20-40%[111-115]. The risk of breast cancer decreases by 6% for each additional hour of physical activity per week lifetime[111]. Adulthood activity reduces risk irrespective of the amount of activity performed before age 20 and is independent of body mass index (BMI)[111]. In the general population, the proposed mechanism by which physical activity affects breast cancer risk is through hormone-related pathways. Other potential biological mechanisms will be discussed in a later paragraph. The association between physical activity and breast cancer risk is most pronounced for postmenopausal and less convincing for premenopausal breast cancer[111,116]. However, the evidence that physical activity may protect against premenopausal breast cancer is increasing[117-119]. Whether a certain intensity of physical activity or physical activity in specific time periods is most effective for lowering breast cancer risk is not yet known.

Only two small studies investigated physical activity and breast cancer risk in BRCA1/2 mutation carriers[18,78]. In a study among 104 carriers with breast cancer[18], cases who participated in sports activity as a teenager had a 10-year delay in breast cancer onset as compared to cases who not did participate in sports activity as a teenager. However, this observation may be attributed to a birth cohort effect as all analyses were univariate. An unmatched case-control study (N=137) investigated recent leisure-time physical activity and breast cancer risk and observed no association[78].

Thus, at present, it is unclear whether an association exists between physical activity and breast cancer risk in BRCA1/2 mutation carriers.

BODY WEIGHT

The evidence that greater BMI or, more specifically, adult weight gain is a cause of postmenopausal breast cancer is convincing in the general population[116,120]. By contrast, obesity may reduce the risk of premenopausal breast cancer[116,120]. The potential biological mechanism for the effect modification by menopausal status will be discussed in the next paragraph.

So far, few studies on anthropometric measures and breast cancer risk in BRCA1/2 mutation carriers have been conducted and showed inconsistent results. The largest study on the association between body weight and breast cancer risk in carriers was a study among 1,073 case-control pairs by Kotsopoulos et al.[121]. They focused on changes in body weight and observed that a loss of at least 10 pounds between ages 18 to 30 years was associated with a decreased risk of breast cancer at ages 30-40. The study was intrinsically stratified on menopausal status of the cases, but it is not quite clear how the menopausal status of the controls was taken into account. The other studies in BRCA1/2 mutation carriers did not adjust for or stratify on menopausal status, which hampers the comparison of results. In a case-only study among 104 carriers, a healthy body weight at menarche and a lighter body weight at age 21 were associated with a significant delay in the age at onset of breast cancer, however, it is not clear whether these findings were adjusted for birth cohort[122]. Nkondjock et al. observed a trend of increased breast cancer risk with increasing weight gain since age 18 and age 30[123]. This effect was independent of physical activity and energy intake. However, the study was unmatched and it was not clear whether the information on body weight was assessed for the prediagnostic period. Chang-Claude et al. observed no association between BMI and breast cancer risk in carriers, but their study was small (24 cases and 8 controls) and in cases BMI was assessed for the postdiagnosic period[124].

Clearly, more data are needed to assess the potential association between body weight and breast cancer risk in BRCA1/2 mutation carriers.

POTENTIAL BIOLOGICAL MECHANISMS OF EFFECTS OF PHYSICAL ACTIVITY AND BODY WEIGHT ON BREAST CANCER RISK

In the general population, the proposed mechanism by which physical activity and body weight affect breast cancer risk is through hormone-related pathways. Estrogens play an important role in the development of breast cancer as is evident from the association with menstrual cycle and reproductive characteristics, such as age at menarche, menopause, and first birth[125]. Increased levels of physical activity have been associated with a later age at menarche, a decreased percent of body fat, and decreased lifetime exposure to estrogens[126,127]. Body size affects pre- and postmenopausal breast cancer risk differently. The increased postmenopausal breast cancer risk observed in obese women is generally explained by the higher rates of conversion of androgenic precursors to oestradiol through increased aromatase enzyme activity in adipose tissue[120]. Furthermore, obesity is associated with increased insulin resistance, resulting in a reduction of circulating sex-hormone binding globulin[128]. Together with the increased formation by the adipose tissue, this leads to an increase in bioavailable fractions of estrogens that can diffuse to target cells. The potential reduced risk of premenopausal breast cancer would arise from an increased tendency for young obese women to have longer menstrual cycles which are more frequently anovulatory as compared to nonobese premenopausal women, resulting in lower serum levels of estrogen and progesterone[128]. For both physical activity and obesity other postulated mechanisms are alterations in metabolism of insulin and insulin-like growth factors (IGFs), altered levels of adipocytokines and immune function[126,127]. Estrogen action is strongly related to the IGF system[129] 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[130,131].
The risk-reducing effects BPSO[42] in BRCA1/2 mutation carriers suggest that hormonal influences are important, despite the fact that BRCA1 breast cancers have a predominantly negative estrogen receptor status[29]. In other words, estrogen may play a role in the early steps of BRCA1-related carcinogenesis[132]. 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[133]. 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[134]. 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[135].

STUDY DESIGN AND POPULATION

The present thesis is based on studies conducted in female BRCA1/2 mutation carriers within the framework of the HEBON study (HEreditary Breast and Ovarian cancer study the Netherlands). The HEBON study is an ongoing nationwide retrospective cohort study with a prospective follow-up planned in the future among members of all families with a BRCA1/2 mutation who were tested after genetic counseling in the Netherlands. The HEBON study was initiated in 1998 by the Departments of Epidemiology and Pathology of the Netherlands Cancer Institute-Antoni van Leeuwenhoek hospital. Figure 4 depicts the design of the HEBON study. The main aim was to investigate cancer risk in familial and hereditary breast and/or ovarian cancer families. In addition, interaction between hormonal and lifestyle risk factors and cancer genes in breast and ovarian cancer development 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).

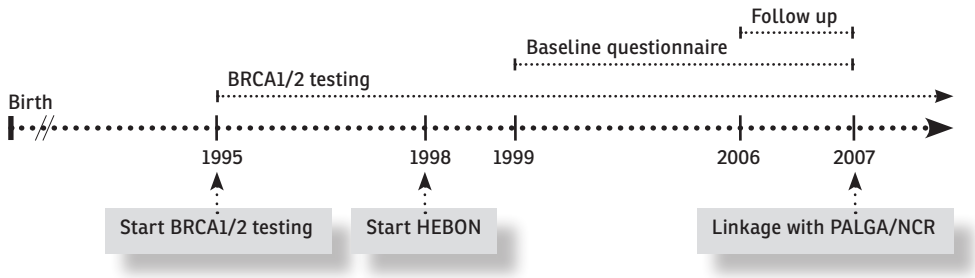

FIGURE 4. Design of the HEBON study

In 2009, an initiative to create a Research Facility for HEBON research was undertaken. 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 hormonal/lifestyle risk factors, treatment, and with disease registries. The HEBON study has been funded by the Dutch Cancer Society and from 2009 onwards by the Dutch Organization for Scientific Research.
BRCA1/2 families were identified through ten centres (nine Clinical Genetic Centres (CGCs)/Family Cancer Clinics and the Foundation for the Detection of Hereditary Tumours (STOET)). The study was approved by the medical ethics committees of all participating centres. Initially, female family members were eligible if they met the following criteria: (a) no personal history of breast and/or ovarian cancer on January 1, 1960, or born after 1960; (b) first- or second-degree relative of an individual diagnosed with breast and/or ovarian cancer and/or a typed carrier (male or female);
(c) age 18 or older at study entry. A self-administered baseline risk factor questionnaire on known and suspected breast cancer risk factors was mailed to eligible family members from 1999 onwards. Family members who had been counseled by the CGC, were invited to participate in the study by their clinical geneticist. All other eligible family members were invited through a so-called contact-person of their family.
If an eligible individual was mentally or physically unable to complete the questionnaire, or had died, a close relative was asked to complete the questionnaire for this individual. This so called proxy questionnaire was a shorter version of the standard baseline questionnaire. In 2006-2007, a follow-up questionnaire was mailed to BRCA1/2 mutation carriers and non-carriers who already had participated between 1999 and 2004. This follow-up questionnaire contained questions on the period since baseline as well as a detailed section on lifetime exposure to diagnostic radiation. This diagnostic radiation questionnaire was also used to replace the existing questions on diagnostic radiation in the baseline questionnaire. From 2006 onwards, the HEBON study started to recruit male carriers and non-carriers (for whom a questionnaire was especially designed), while proxy questionnaires were no longer used. All participants (or their proxies) signed an informed consent form. Information on cancer history and prophylactic surgery was either self-reported for the period not covered by the registries (<1989) and/or collected through the Netherlands Pathology Database (PALGA)[136] and the Netherlands Cancer Registry (NCR) (see Figure 4). In the studies described in this thesis, ninety-five percent of breast cancers diagnosed after 1988 were confirmed by PALGA/NCR. Information on deaths was retrieved through municipal registries.
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). However, the power among non-carriers is low. The study of gene-risk factor interactions in epidemiological studies may help identify potential biological pathways. A problem in most studies examining the influence of risk factors on breast cancer risk

in BRCA1/2 mutation carriers is that these studies 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 take 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 section on prophylactic surgery) 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[137]. A difference between a traditional retrospective cohort study and our retrospective cohort studies within HEBON is the fact 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 1995 had been diagnosed with breast cancer in the past, prevalent cases are included, resulting in potential survival and/or recall bias. To reduce potential survival bias a priori, information on deceased (obligate) carriers who had died before study entry was collected through proxies. 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. Another important issue is potential selection bias based on the DNA test (or: testing bias); women were selected from high-risk families qualifying for genetic testing and the disease status may therefore have increased the likelihood of ascertainment leading to an oversampling of affected women. To correct for this potential bias, analyses were performed using the weighted regression approach described by Antoniou et al.[138]. By this procedure, relative risks are typically shifted away from the null value (RR=1) at the cost of some power (wider 95% confidence intervals).

AIMS OF THIS THESIS

The aim of the studies described in this thesis is to contribute to the increasing understanding of modifying risk factors in BRCA-associated breast cancer. Additionally, the results described in this thesis will hopefully provide a target to add to breast cancer prevention in this high risk population, and expand the scope of genetic counseling resulting in improved information for and education of BRCA1/2 mutation carriers. More specifically, the aims of this thesis are:

- to assess the association between breast cancer risk and exposure to ionizing radiation from diagnostic procedures, like fluoroscopies, chest X-rays, and mammograms, in different age periods in BRCA1/2 mutation carriers in a large European collaborative study (GENE-RAD-RISK) including carriers from France, the United Kingdom, and the Netherlands
- to examine whether a potential association between diagnostic radiation and breast cancer risk in BRCA1/2 mutation carriers can be (partially) attributed to non-differential and/or differential misclassification bias (recall bias)

- to investigate whether physical activity is associated with breast cancer risk in BRCA1/2 mutation carriers. Moreover, we wanted to investigate whether a potential association was related to specific types and/or dimensions (aspects) of physical activity
- to investigate whether different anthropometric measures are associated with breast cancer risk in BRCA1/2 mutation carriers. More specifically, we were interested in potential effect modification by menopausal status, which is known to exist in the general population
- to examine potential survival bias in the studies on diagnostic radiation, physical activity, and body weight, due to the inclusion of prevalent cases

OUTLINE OF THIS THESIS

Chapter 2 of this thesis describes a European collaborative study (GENE-RAD-RISK study) in which we investigated the association between lifetime diagnostic radiation history and breast cancer risk in 1,993 BRCA1/2 mutation carriers from France, the United Kingdom and the Netherlands. A unique feature of this study was the estimation of a cumulative breast dose score from various diagnostic radiation exposures. An important limitation that was repeatedly highlighted in the previously described diagnostic radiation studies in BRCA1/2 mutation carriers is potential recall bias. However, so far, no studies have investigated this critical issue further. Therefore, within the Dutch cohort of the GENE-RAD-RISK study, we conducted 2 methodological studies on test-retest reliability and validity of self-reported diagnostic radiation history, of which the results are described in **chapters 3** and **4**. **Chapters 5** and **6** describe studies on two of the more modifiable lifestyle factors and breast cancer risk in BRCA1/2 mutation carriers. **Chapter 5** describes the first large study specifically investigating lifetime physical activity and breast cancer risk in 1,026 Dutch BRCA1/2 mutation carriers. In **chapter 6** we report on a study in which we investigated various anthropometric measures, like BMI at age 18 and adult weight change, and breast cancer risk in 980 Dutch BRCA1/2 mutation carriers. The body weight analyses were stratified on menopausal status. An important feature of the physical activity and body weight studies was the fact that we adjusted both analyses for the other risk factor so that the observed associations were mutually independent. An important feature of the studies described in chapters 2, 5, and 6 was the investigation of potential survival bias and the application of the weighted cohort approach to correct for potential testing bias. In **chapter 7**, the main findings are described, methodological strengths and weaknesses are discussed, various aspects of the conducted studies (e.g. biological mechanisms and barriers encountered during data collection) are described, conclusions are drawn, and implications for clinical practice and recommendations are given.

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