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Assessment of risk and risk factors for hereditary breast cancer & cardiovascular disease: Opportunities for diagnosis and prevention

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List of Acronyms

Bio-ADM	Biologically active adrenomedullin
BOADICEA	Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm
BMI	Body mass index
BOCRMC	Breast and Ovarian Cancer Risk Management Clinic
BPM	Bilateral prophylactic mastectomy
CI	Confidence interval
CVD	Cardiovascular disease
DCIS	Ductal carcinoma in situ
FRA-BOC	Familial Risk Assessment Tool – Breast and Ovarian Cancer
G3	Grade three
GWAS	Genome wide association studies
IBIS	International Breast Cancer Intervention Study
LIBRE	Lifestyle intervention trial in women at increased risk for breast and ovarian cancer
MD	Mediterranean Diet
MET	Metabolic equivalent of task
MRI	Magnetic resonance imaging
OPG	Osteoprotegerin
OR	Odds ratio
PBSO	Prophylactic bilateral salpingo-oophorectomy
sRANKL	Soluble receptor activated nuclear factor-κB ligand
ROC	Receiver operating characteristic
SNP	Single nucleotide polymorphism
USA	United States of America

Abstract

Women who inherit a deleterious *BRCA1* or *BRCA2* mutation are at an increased lifetime risk of developing breast cancer, with estimates ranging from 69% to 72% by the age of 80. Although genetic testing allows for the identification of high-risk individuals, prevention options for these women are limited to bilateral prophylactic mastectomy. Although it is established that modifiable lifestyle factors, such as physical activity and maintaining a healthy weight, can significantly lower the risk of breast cancer in the general population, it remains unclear whether these factors are similarly effective in preventing *BRCA*-associated breast cancer.

The first study evaluated the relationship between physical activity during adolescence and subsequent breast cancer risk in *BRCA1/2* mutation carriers. The findings of this analysis indicate that moderate physical activity during the ages of 12 to 17 may lead to a 38% lower risk of premenopausal breast cancer in women with *BRCA* mutations.

Women with a history of breast cancer are at a considerable risk of mortality from cardiovascular disease (CVD) due to pre-existing vulnerability and the negative impact of cancer treatments on cardiovascular health. Simultaneously, research conducted on both mice and humans has indicated a link between severe cardiac events, such as myocardial infarction, and the promotion of breast cancer progression, which includes a rise in cancer-related deaths. Common risk factors, including age, obesity, and unhealthy eating habits, further exacerbate the risk of both breast cancer and CVD in susceptible women. Thus, it is imperative to enhance cardiovascular care in women at increased risk for breast cancer and breast cancer survivors to reduce their cancer risk and manage cardiovascular risk factors effectively. Recent studies have highlighted the crucial role of *BRCA* genes in regulating the survival and function of cardiomyocytes, with loss of function increasing susceptibility to cardiac damage, rendering individuals

with *BRCA* mutations more vulnerable to CVD. Currently, no established recommendations exist for screening, monitoring, and managing CVD risk among the general oncology population. Given the increased risk of both breast cancer and CVD, *BRCA1/2* mutation carriers may represent a suitable population to monitor and control CVD risk factors and study surrogate markers for CVD.

The objective of the second study was to examine the relationship between biologically active Adrenomedullin (bio-ADM), a potential biomarker for subclinical cardiac dysfunction, and cardiovascular risk factors in a group of *BRCA* mutation carriers. Women who had metabolic syndrome exhibited a 22-fold greater chance of having high bio-ADM levels. Higher bio-ADM levels were associated with reduced cardiorespiratory fitness, various obesity indices and smoking.

The management of breast cancer risk in *BRCA1/2* mutation carriers is well-established, but the optimal approach for managing the cancer risks of women with a strong family history but no known familial mutation for breast cancer remains uncertain.

Therefore, we evaluated the clinical outcomes of high-risk women who underwent breast cancer screening services at a specialty clinic in Melbourne, Australia, and described the incidence of breast malignancies and the uptake of preventive options for both mutation carriers and noncarriers.

Between 2010 and 2018, breast cancer screening was conducted on at least one occasion for a total of 206 mutation carriers and 305 noncarriers at high risk for breast cancer whose median age was 37 years. High risk for breast cancer was captured by the Familial risk assessment - breast and ovarian cancer (FRA-BOC) online tool. During a median follow-up of 34 months, 15 (seven invasive) breast cancers were detected in mutation carriers and seven (six invasive) in noncarriers. The median size of invasive breast cancers was 11 mm (range: 1.5-30 mm), with the majority being axillary

node negative. The study found that using a cut-off of 25% lifetime risk on Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) as an inclusion criterion for intensified breast cancer screening would have excluded 77.6% of noncarriers, despite the fact that many of these women may have a high risk of developing breast cancer. Even though six out of seven women without mutations who had screen-detected breast cancers met the old inclusion criteria provided by the FRA-BOC online tool, they did not meet the BOADICEA threshold of a lifetime risk of at least 25%. Four of these women were diagnosed with breast cancer before the age of 50. The study suggests that breast cancer screening was effective in detecting early-stage cancers, and there is a higher incidence of events in young noncarriers compared to the general population. As a result, noncarriers require continued management through a specialty clinic. However, to improve cancer detection rates and screening efficacy, it is necessary to enhance risk assessment and inclusion criteria for high-risk noncarriers.

In summary, these studies highlight the importance of ongoing research into optimal approaches for managing individuals with *BRCA* mutations or a significant family history of breast cancer. Identifying effective prevention strategies and managing cardiovascular risk factors can improve the quality of life and long-term health outcomes for these individuals.

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1 Introduction

1.1 Overview of breast cancer risk and management among *BRCA1/2* mutation carriers

The *BRCA1* and *BRCA2* genes are tumour suppressor genes. When working properly, they provide instructions for making proteins that are involved in cell cycle control and DNA repair. Mutations in these genes can disrupt the normal function of the proteins they produce, leading to an increased risk of developing certain types of cancer (1).

In particular, inheritance of a mutation in *BRCA1* or *BRCA2* is associated with a very high risk of developing breast cancer, estimated at 72% and 69%, respectively by age 80. Breast cancer risk in a population of 3,886 *BRCA1/2* mutation carriers with ethnic diversity was studied prospectively. The study found that the risk of breast cancer rose sharply in early adulthood and then stabilized at a relatively constant rate. The highest risk of breast cancer occurred in the 30s for *BRCA1* mutation carriers and in the 40s for *BRCA2* mutation carriers (2).

Women with *BRCA*-associated breast cancers have an increased risk of developing a second ipsilateral (3) or contralateral (4) breast cancer. *BRCA1*-associated breast cancers tend to be basal-type, high grade and hormone receptor-negative, whereas *BRCA2*-associated breast cancers resemble sporadic breast cancers, i.e. luminal B breast cancers that are predominantly hormone receptor-positive (5, 6).

Individuals who are heterozygous for a *BRCA* mutation are carriers of the mutation, but may not necessarily develop cancer. This is because the wild-type copy of the gene is still able to produce the *BRCA* protein, which can partially compensate for the loss of function caused by the mutated copy.

Mutations affecting both copies (biallelic) of the *BRCA2* gene can lead to the clinical presentation of Fanconi anaemia type D1 and significantly increase

the risk of developing childhood cancers. Notably, biallelic mutations in *BRCA1* have been rarely reported and are most likely embryonic lethal in the majority of cases (7).

Genetic penetrance refers to the proportion of individuals with a particular genetic mutation who actually develop the associated disease. In the case of the *BRCA* genes, the genetic penetrance is not 100%, i.e., not all individuals who inherit a *BRCA* mutation will develop cancer.

Variations in penetrance estimates have initially been attributed to the method of ascertainment of the families under study; however, regional differences in penetrance of an inherited *BRCA1* or *BRCA2* mutation and an increasing trend in penetrance among individuals carrying these mutations born in later generations compared to those born earlier have prompted the search for factors other than the gene itself which may be influencing the risk of cancer in susceptible women (8, 9). To date, both genetic and non-genetic factors have been suggested to influence breast cancer risk in *BRCA1* and *BRCA2* mutation carriers. Part of the variation in risks may be explained by the position of the mutation within each gene, by other risk-modifying genes, by the additional effect of common single nucleotide polymorphisms (SNPs) across the genome, and by environmental factors (10, 11).

Primary prevention of breast cancer in susceptible women is limited to bilateral prophylactic mastectomy (BPM). This prophylactic measure can decrease the risk of breast cancer by up to 95% (12). Despite the strong protection offered by this procedure, the uptake varies greatly between European countries with rates between 0.5% and 48.5% in the International *BRCA1/2* mutation carrier Collaborative Study (personal communication), underlining a remarkable impact of cultural factors and subjective preferences of healthcare professionals on their patients' choice (13).

Alternative non-surgical chemopreventive options in *BRCA1/2* mutation carriers are based on an interruption of the oestrogen-signalling pathway (e.g. through tamoxifen or aromatase inhibitors) and, in *BRCA1* mutation

carriers, the receptor activated nuclear factor-κB ligand (RANKL)-driven progesterone signalling (e.g. through denosumab), yet their complete effectiveness still remains to be elucidated (14) and is under active study (15). Uptake of chemoprevention with selective oestrogen receptor modulators such as tamoxifen or with aromatase inhibitors is low. To date, no large-scale study has evaluated the effectiveness of tamoxifen or aromatase inhibitors for the primary prevention of *BRCA*-associated breast cancer. Instead, the recommendation is derived from studies conducted predominantly in noncarriers at increased risk for breast cancer (16).

Women who are not (yet) willing to proceed with preventive surgery can also rely on specialized surveillance programs aimed at early detection of breast cancer, including frequent screening of the breasts through breast ultrasounds, breast magnetic resonance imaging (MRI) or mammograms (16). Whether enhanced screening with breast MRI is a viable alternative to prophylactic BPM is unknown; no studies have compared mortality with breast MRI screening vs. BPM specifically in *BRCA1/2* mutation carriers.

The varying penetrance estimates along with the limited chemopreventive options indicate the need to identify additional options that may help modify breast cancer risk. *BRCA1/2* mutation carriers have strongly expressed their preference for less invasive prevention options (17); however, further investigations are needed before one can provide effective and safe recommendations to reduce breast cancer risk in *BRCA1/2* mutation carriers without resorting to surgery.

1.1.1 Impact of modifiable risk factors on (BRCA-associated) breast cancer

Decades of epidemiologic research have witnessed a burgeoning interest in the study of lifestyle and environmental breast cancer risk factors among the general population, including reproductive history, use of hormones,

(changes in) body composition (across the lifespan), and alcohol intake, each explaining a modest proportion of the variation in breast cancer risk (18). However, when combined, these environmental risk factors have a substantial impact on disease risk. In fact, according to data derived from the general population in Western countries, almost one in three breast cancer cases could be prevented by adapting healthy lifestyles (19).

More recent data combining the impact of low penetrant SNPs with epidemiological risk factors have helped to understand the distribution of breast cancer risk across populations. These models have shown that lifestyle modifications could have a greater absolute effect if targeted to women at higher absolute risk, including women at higher-than-average risk due to their family history or genetic susceptibility. In this respect, it is encouraging that even women at highest breast cancer risk due to nonmodifiable factors, who sustained a healthy weight, did not consume alcohol or tobacco and did not use hormone replacement therapy, had risks comparable to the average in the general population. Recent findings demonstrated that a greater proportion of preventable cases of breast cancer would be observed in women with elevated levels of risk due to genetic and nonmodifiable factors. This suggests that interventions aimed at modifying risk factors, which may not be feasible for the entire population due to costs and other limitations, could be directed towards high-risk groups to achieve a greater reduction in the incidence of breast cancer (18, 20).

We have published a review of the epidemiologic evidence on the association between modifiable risk factors and (hereditary) breast cancer risk (18).

While various reproductive and hormonal factors have shown to impact *BRCA*-associated cancer risk, the risk-reducing potential of modifiable lifestyle factors, including body composition, physical activity, and diet, are yet to be elucidated in this population.

1.1.2 Physical activity and breast cancer risk in BRCA1/2 mutation carriers

The role of physical activity in the aetiology of breast cancer among average-risk women has been studied extensively and physical activity remains the most important modifiable risk factor (18). The evidence unequivocally supports an inverse association between physical activity and breast cancer, with a reduction of 25%-30%, comparing the most versus least active women (21).

For women at high-risk due to an inherited *BRCA1/2* mutation, there is also emerging evidence suggesting a protective role of exercise.

Five epidemiological studies have evaluated the effect of physical activity specifically on *BRCA*-associated breast cancer risk, but with mixed results.

King *et al.* were the first to report a significant delay in breast cancer onset among 104 *BRCA1/2* mutation carriers who were physically active as teenagers compared to those who were not (22). A second study (n = 137) found no association between levels of adult physical activity and subsequent breast cancer risk (23). In a retrospective cohort study (n = 725), Pijpe *et al.* reported a significant 42% reduction in risk with increasing levels of physical activity prior to, but not after, age 30 (24). In our retrospective observational study of 68 *BRCA1/2* mutation carriers, study participants who indicated higher physical activity levels during adolescence had a significantly lower cancer prevalence (25). In a prospective cohort of 15,550 women with a family history of breast cancer, including 659 *BRCA1* and 526 *BRCA2* mutation carriers, baseline recreational activity in the highest four quintiles compared to the lowest quintile was associated with a 20% decrease in breast cancer risk. However, in this analysis, adolescent recreational physical activity was not associated with subsequent breast cancer risk (26).

Collectively, these epidemiologic studies suggest a protective role of physical activity in the development of *BRCA*-associated breast cancer.

However, the optimal exercise type, timing, and dose of exercise remain uncertain. Given the mixed results with regard to adolescent physical activity and subsequent breast cancer risk, it is not clear whether physical activity during adolescence is protective against *BRCA*-associated breast cancer or only delays development of disease.

1.2 Cardiovascular disease risk assessment in *BRCA1/2* mutation carriers

1.2.1 The link between breast cancer and cardiovascular disease

As breast cancer outcomes improve with earlier detection and advances in treatment, cardiovascular disease (CVD) has become an important cause of morbidity and mortality among (early) breast cancer patients (27). In fact, CVD overtakes breast cancer as the primary cause of mortality in breast cancer survivors eight years after the initial diagnosis (28, 29). CVD can be caused or accelerated by a variety of breast cancer treatments, i.e. due to exposure to anthracyclines, Her2-targeted agents, chest radiation therapy and long-term oestrogen suppression (30, 31). Concomitantly, studies in mice and humans have suggested an association between serious cardiac events, e.g. myocardial infarction, and promotion of breast cancer progression, including an increase in cancer-specific mortality (32). Additionally, there is a significant overlap between CVD and breast cancer, including a number of common risk factors, i.e. aging, physical inactivity and metabolic syndrome, suggesting a shared biology (33). Cancer and hypertension often coexist in the same individuals. A large observational cohort study has revealed that hypertension is the most prevalent comorbidity among cancer patients, with a reported prevalence of 38%. This study was conducted before the widespread introduction of many targeted therapies that are associated with hypertension. Therefore, the reported prevalence is expected to be lower than

the current prevalence of hypertension among cancer patients (34). A meta-analysis comprising of 30 prospective studies found that postmenopausal women with hypertension had a 20% higher risk of developing breast cancer (34). A retrospective analysis of more than 25,000 adult cancer patients in the United States of America (USA) revealed that nearly one-third of them developed de novo hypertension during follow-up. Anticancer therapy was found to be associated with a 2- to 3.5-fold higher risk of developing hypertension (35). Thus, cardiovascular risk factors are more pronounced in patients with a history of breast cancer than in age-matched, cancer-unaffected women (36, 37), and anticancer drugs may result in deterioration of pre-existing cardiovascular conditions.

With the exception of recurrent cancer, CVD is the most important competing risk of death in women with a history of breast cancer due to baseline predisposition and short- and long-term adverse cardiovascular toxicities associated with cancer therapies. It is therefore crucial to optimize cardiovascular care and risk profiles in breast cancer patients and survivors, and make sure that improvements in cancer survival do not come at the cost of cardiovascular health (34).

1.2.2 Screening programs for cardiovascular risk in breast cancer survivors and surrogate markers for CVD

In order to prevent CVD, it is crucial to identify high-risk individuals long before the development of cardiac injury.
Ideally, all patients should be monitored for pre-existing cardiovascular risk factors before initiating antineoplastic therapy, especially in those who will be exposed to cardiotoxic and hypertensive-inducing agents. Collaborative and proactive management decisions should be made in a multidisciplinary team involving cardio-oncologists, with the goal of achieving a balanced approach that minimizes any potential delay in initiating necessary

anticancer therapy. The objective should be to minimize the risk of hypertension-related end-organ damage and prevent the need for future anticancer therapy interruption or dosage reduction due to de novo hypertension, a deterioration of previously well-controlled hypertension or (major) cardiac events (34).

Current recommendations primarily concentrate on screening for cardiovascular issues and monitoring cardiac function before or during cancer treatment. However, as cancer treatments advance and lead to improved cancer outcomes, there is an increasing need for reliable guidelines to manage and monitor CVD and associated risk factors in cancer survivors over the long term. The long latency period of approximately five to seven years between the initial diagnosis of breast cancer and manifest CVD (38, 39) provides a window of opportunity to identify and treat CVD risk factors before any clinical signs or symptoms become evident.

One of the barriers to improving cardiovascular disease outcomes in breast cancer survivors is the inadequacy of common screening tools: The traditional Framingham Risk Score significantly underestimates a breast cancer survivor's risk of developing CVD (37, 40), lending urgency to find a more suitable method to assess CVD risk in these at-risk patients (41).

Non-invasive biomarkers incorporating different pathophysiological processes of CVD have become a major focus of attempts to predict cardiovascular events among the general population (42). The value of blood-based biomarkers to identify preclinical CVD in breast cancer survivors is not known.

Predisposition for atherosclerosis has been shown to be a surrogate for overall CVD risk and can be non-invasively assessed by the use of structural and functional vascular biomarkers. Structural biomarkers include measures of arterial stiffness, thickness, and diameter, while functional biomarkers assess blood flow, endothelial function, and other physiological parameters. Adrenomedullin (ADM), a natriuretic and diuretic peptide, is produced in

response to mechanical stretch and thus reflects vascular changes. ADM represents a promising biomarker of vascular health, and it becomes elevated years before the onset of non-communicable diseases (43). In particular, elevated ADM levels in healthy individuals are strongly associated with subsequent CVD and cancer development, as well as premature mortality (44). Lifestyle interventions have the potential to reverse adverse vascular alterations, thus, it has been shown that ADM is responsive to lifestyle modifications, including physical activity and diet (45-48).

1.2.3 Role of the BRCA genes in cardiovascular health

With respect to developing screening programs for secondary prevention of CVD, it remains to be determined who, when and how to screen. The aim of these programs is to identify women at significant risk that are most in need for lifestyle modifications or early treatment, in order to shift their trajectories away from developing manifest CVD. Targeting high-risk groups is a common approach to developing screening programs.

Having a risk of 69-72% of developing breast cancer and a risk of 17-44% for developing ovarian cancer by age 80 years (2), *BRCA1/2* mutation carriers are exposed to cancer treatments and prophylactic bilateral salpingo-oophorectomy (PBSO) with detrimental short- and long-term adverse cardiovascular toxicities (49).

BRCA1/2 mutation carriers face an exceptional risk for CVD due to a combination of treatment-related and hormonal factors. Firstly, *BRCA1/2* mutation carriers are at increased risk for developing breast cancer at a younger age than the general population, with a median age of onset at 51 years compared to 64 years (2). They also have a high risk of developing contralateral (4) or ipsilateral cancer (3). Secondly, *BRCA*-associated cancers are often aggressive in nature (e.g., G3 cancers, basal-like disease in *BRCA1* mutation carriers and luminal B tumours in *BRCA2* mutation

carriers) (5, 6), and may therefore require potentially cardiotoxic chemotherapy. Thirdly, for those with ER-positive breast cancer, extended adjuvant endocrine therapy can be beneficial, especially in premenopausal women (50). Additionally, *BRCA1/2* mutation carriers are advised to undergo PBSO after child-bearing age. However, long-term oestrogen deprivation in women undergoing PBSO can lead to an increased risk of CVD by two- to threefold compared to women of the same age without surgical menopause (51, 52).

One and a half decades after the discovery of the *BRCA* genes, reports have suggested that *BRCA1/2* mutation carriers have a reduction in life expectancy of approximately four to six years compared to the general population, even if they were never diagnosed with cancer (53). Although the causes of death were not recorded, this has prompted research investigating the role of the *BRCA1/2* genes in non-cancer conditions including cardiovascular health.

Preliminary evidence indicates that *BRCA1/2* mutation carriers are more prone to cardiovascular disease both at baseline and in response to cancer treatments (54-58).

Recent studies propose that the *BRCA* genes regulate cardiomyocyte survival and function, and that loss of function raises the vulnerability to cardiac damage (56-58). Experimental findings in mice have shown that *BRCA1* limits endothelial cell apoptosis, restores endothelial function, and attenuates atherosclerotic lesion development (59). Moreover, loss of *BRCA2* has been associated with increased susceptibility to doxorubicin-induced heart failure (60).

It is crucial to thoroughly evaluate cardiovascular risk factors, and the possible adverse effects on organs before, during, and after anticancer treatment. At present, there are no established recommendations for screening, monitoring, and managing CVD risk among the general oncology

population. However, the necessity for developing such guidelines is evident from both clinical and scientific perspectives.
Therefore, being at increased risk for breast cancer and CVD, *BRCA1/2* mutation carriers might be a good target group to monitor and control CVD risk factors and to study a surrogate marker for cardiovascular disease. The results obtained from these analyses could aid in comprehending and resolving the intricate and interconnected concerns associated with breast cancer and CVD in the future (61).

1.3 Clinical management of women at high risk for breast cancer

It is estimated that about 5%-10% of breast cancer cases are thought to be hereditary (62). Up to 25% of inherited cases of breast cancer are caused by mutations in a small number of genes that are rare but highly penetrant, including *BRCA1*, *BRCA2*, *CDH1*, *PALB2*, *PTEN*, *STK11* and *TP53*. These mutations can increase the lifetime risk of developing breast cancer by up to 80%. Approximately 2%-3% of cases of breast cancer can be attributed to mutations in rare, moderate-penetrance genes such as *ATM* or *CHEK2*, which are associated with a two- to threefold increase in risk. Current prediction models suggest that there are unlikely to be any additional high-penetrance genes that have not yet been identified. While research into common, low-penetrance alleles has identified a number of suggestive SNPs that contribute to breast cancer risk, the individual risk associated with each SNP is relatively low. However, a combination of these variants may result in a considerable increase in breast cancer risk. Next-generation sequencing offers new possibilities for risk assessment. It is anticipated that future studies will provide more clarity on the function of modifier genes and elucidate the combined impact of mutations or polymorphisms in multiple

genes, which may work together in an additive or synergistic manner (63). Currently, targeted multigene panel testing is recommended if there is a high suspicion of a genetic predisposition due to personal history or family history. While clear clinical guidelines exist for individuals with mutations in high-penetrance genes, both preventive strategies and risk factors for the development of breast cancer have not been investigated to the same extent in high-risk women who tested negative for *BRCA1/2* mutations or where no heritable germline mutation has been identified (64).

This group represents a significant population of at-risk women, who are currently under-studied and under-serviced although they outnumber *BRCA1/2* mutation carriers. For women where no heritable germline mutation has been identified, there currently is no clear consensus on the best practices in their risk assessment, management and care with regard to intensified breast cancer screening and risk-reducing options (64).

1.3.1 Risk-adapted breast cancer screening

Due to ongoing debates about screening recommendations for women at an average risk of developing breast cancer, researchers have been exploring personalized screening approaches. The objective is to customize screening recommendations, including appropriate starting age, screening frequency, and protocols, based on an individual's estimated risk level (65).

The purpose of breast cancer risk models is to predict the probability of a woman developing invasive breast cancer over a defined timeframe, such as ten years or her lifetime.

These models have been developed and validated for use at the population level and are now recommended for use in clinical practice to assess individual patients. The variables considered in these risk models vary in number and weighting. Although age is a significant predictor in all models,

other factors such as family history, genetic carrier status, breast density, and lifestyle risk factors may also be considered (66).

Breast cancer risk can be assessed using empirical models and genetic models. Empirical models are based on clinical and lifestyle risk factors such as age, family history of breast cancer, age at menarche, age at first childbirth, menopausal status, and use of hormone replacement therapy. These models estimate an individual's risk based on the prevalence of these factors in the general population and the strength of their association with breast cancer. Examples of empirical models include the Gail model, Tyrer-Cuzick model, and the Breast Cancer Surveillance Consortium risk calculator. Genetic models, on the other hand, incorporate genetic information into the assessment of breast cancer risk. These models use information on variants in specific genes that have been associated with an increased risk of breast cancer, such as *BRCA1* and *BRCA2*. Genetic models also take into account the presence of other genetic variants associated with breast cancer risk, including SNPs. Examples of genetic models include the BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm) and BRCAPRO models (66).

These widely used risk prediction models exhibit comparable accuracy and strong discrimination when evaluated in large populations (67).

Although breast cancer risk assessment tools are effective when used at the population level, their performance at the individual level and the effects of varying thresholds for identifying high-risk individuals (Australia: lifetime risk ≥25% according to Cancer Australia; Germany: lifetime risk ≥30% according to the German Consortium for Hereditary Breast and Ovarian Cancer, United Kingdom: lifetime risk ≥30% according to the National Institute of Health Care Excellence; USA: lifetime risk ≥20% according to the American Cancer Society) have been largely overlooked. With the shift towards a precision medicine approach in healthcare, these risk models are

now more frequently used to identify women who may benefit from chemoprevention and supplemental MRI screening (66).

Evaluating risk accurately requires familiarity with each model and the consideration of multiple factors. The selection of the model depends on the purpose of the assessment. For instance, the Gail model is more effective in predicting the potential benefits of chemoprevention, while the BRCAPRO, Claus and Tyrer-Cuzick models are more useful in assessing the efficacy of breast MRI as a supplemental screening tool (68, 69).

Despite the availability of multiple risk estimation models, there is no consensus on which model is the most accurate and reliable. When comparing different models within a cohort, there is very little agreement in the assigned risk estimates (66).

Ozanne *et al.* conducted a study using the BRCAPRO, Claus, and Tyrer-Cuzick calculators to determine the eligibility of women for breast MRI screening based on a lifetime risk of ≥20%. Using data from 10,000 women, the Tyrer-Cuzick model identified 5.6% of women as eligible, compared to 0.4% and 0.9% identified by BRCAPRO and Claus models, respectively. Only 0.2% of the study population was found to be eligible by all three methods (70).

Quante *et al.* evaluated the performance of the risk models IBIS and BOADICEA to determine eligibility for MRI screening (lifetime risk of ≥20%). Based on data from 1,764 women, IBIS identified 59.3% of women as eligible, compared to 20.1% identified by BOADICEA (71).

Moreover, each model is designed for specific patient populations, and most of these models have been validated on predominantly white women living in Europe or the USA and may not perform well in other subgroups (66, 69). In order to decrease misclassification bias and provide a more precise risk evaluation for individual patients, current research is exploring the potential of combining traditional risk models to create a "hybrid" model (72). Additionally, recent studies have recommended the inclusion of SNPs in risk

models as they have been found to be significant independent predictors of breast cancer risk and could enhance the accuracy of the models (72).

1.3.2 Multimodality screening for breast cancer and preventive measures

Multimodality breast cancer screening refers to the use of multiple imaging techniques to detect breast cancer. It involves combining different imaging modalities, such as mammography, ultrasound, and MRI, to provide a more accurate and comprehensive assessment of breast tissue.
Mammography is the primary imaging modality used for population-based breast cancer screening, but it has limitations, particularly in women with dense breast tissue. Ultrasound and MRI are often used in addition to mammography to improve the detection of breast cancer in these women or in those at high risk of breast cancer due to genetic mutations or personal or family history.

Magnetic Resonance Imaging

MRI is highly sensitive, but it also has a relatively high recall rate of around 10%. A recent systematic review of eleven published studies found that the sensitivity of mammography was 0.75, while MRI had a sensitivity of 0.92. However, both methods had similar levels of specificity, with values of 0.71 and 0.72, respectively (73). The weighted area under the summary receiver operating characteristic (ROC) curves was significantly higher for MRI, at 0.93, compared to 0.73 for mammography. Screening guidelines for women at high risk of breast cancer often recommend the use of MRI, as it can detect smaller cancers at an earlier stage. It is particularly useful for detecting breast cancer in individuals who carry pathogenic variants in the *BRCA1* and *TP53* genes. However, the main drawback of MRI is its cost, as well as the time taken to perform the examination and the use of a contrast agent, which carries a risk of toxicity. Alternatively, abbreviated or fast MRI, which takes

about one-tenth of the time of standard MRI, has been found to be equally effective and may be preferred in the future (68).

Chemopreventive agents

Endocrine preventive therapy guidelines for *BRCA1* and *BRCA2* mutation carriers are currently absent because there have been no chemoprevention trials conducted specifically for this population to inform such recommendations. The available data from observational studies and secondary analyses are restricted, and tamoxifen and other selective oestrogen receptor modulators do not seem to significantly reduce the incidence of breast cancer related to *BRCA1* mutations. This is attributed to the high prevalence of triple-negative breast cancer in individuals with *BRCA1* mutations. International guidelines recommend chemoprevention for high-risk women who do not carry a *BRCA1/2* mutation to reduce their risk of developing breast cancer (74, 75). However, it is essential to weigh the benefits against potential adverse effects, which can be problematic in some women, depending on the drug. Mocellin *et al.* (76) conducted a network meta-analysis of randomized controlled trials for chemoprevention drugs for breast cancer. This analysis enabled the examination of efficacy (how well the drug works) and acceptability (using side effects data as a measure of toxicity) simultaneously to determine the most successful drugs in terms of meeting both requirements. The study results demonstrated that aromatase inhibitors (anastrozole and exemestane) and lasofoxifene had a high level of evidence, while arzoxifene, raloxifene, tamoxifen, and tibolone had a moderate level of evidence in terms of efficacy and acceptability in breast cancer chemoprevention. Tamoxifen is the only effective chemoprevention drug in premenopausal women, and no drugs have been proven to be effective in reducing oestrogen receptor negative disease (76). Nevertheless, identifying the individuals who are most likely to benefit from chemoprevention drugs remains a challenge, and ongoing research is

currently being conducted in this area. International guidelines recommend anastrozole for postmenopausal women and tamoxifen for premenopausal women (68). Tamoxifen treatment for five years can reduce the incidence of oestrogen receptor positive breast cancer by 38%, while anastrozole reduces breast cancer incidence in high-risk postmenopausal women by 53%. However, the uptake of chemoprevention remains low (66).
The role of the RANKL inhibitor denosumab in preventing *BRCA1*-associated breast cancer in individuals between 25 and 50 years of age is currently being evaluated in an international phase tree trial (15).

Bilateral prophylactic mastectomy

BPM has been shown to reduce the risk of breast cancer by 95% in *BRCA1/2* mutation carriers, and any risk-reducing surgery has been found to lower mortality in this population. However, the benefit of BPM declines rapidly with increasing age at surgery. A study in Australia that investigated data between 2001 and 2005 found that 5.2% of *BRCA* mutation carriers underwent BPM and 16.3% underwent PBSO at three-year follow-up from genetic testing. BPM uptake rates vary widely by country, with the US having the highest rate at 36.3% (66). The public disclosure by Angelina Jolie of her *BRCA1* mutation and decision to undergo BPM led to a significant increase in genetic testing and mastectomy rates globally since 2013 (77). In the United Kingdom, the uptake of BPM was largely dependent on lifetime risk estimates of developing breast cancer, with higher risk women being more likely to undergo surgery (68).
A prospective cohort study of 550 women from ten European countries that provided genetic counselling services found that risk-reducing mastectomy was remarkably effective in decreasing the likelihood of developing breast cancer. No breast cancers were discovered during 3334 women-years of follow-up, whereas 34 breast cancers were expected based on life tables (78).

Still, the recommendation for risk-reducing surgery should be restricted to individuals at the highest risk of developing breast cancer (68).

1.3.3 Risk management practices at the Royal Melbourne Hospital in Melbourne, Australia

Medicare Australia has provided subsidy for breast MRI screening for annual surveillance since February 1, 2009, for women under the age of 50 who are at a high risk of developing breast cancer. This Australian Government initiative is aimed at diagnosing breast cancer in asymptomatic women who are a part of an organized surveillance program and are at a high risk of developing breast cancer. Women under the age of 50 who have tested positive for a genetic mutation that puts them at risk of developing breast cancer, or those who are categorized as high-risk for breast cancer (lifetime risk of ≥25%) are entitled to subsidized breast MRI screening access (66).
Until 2021, Familial Risk Assessment – Breast and Ovarian Cancer (FRA-BOC) was an online tool used in Australia by healthcare professionals to assess an individual's risk of developing breast and ovarian cancer based on family history.
Category three of the tool captured "high-risk" individuals with a lifetime risk of 25% or greater. However, this category was quite expansive and could result in underestimating risk in small families with few at-risk relatives or overestimating risk in large families with multiple at-risk relatives. Additionally, the online tool did not consider tumour pathology (66).
In contrast, Cancer Australia currently recommends using a more individualized approach to breast cancer screening based on a combination of validated risk models that consider family history, lifestyle factors, and tumour pathology without specifying which empirical model to use for calculating an individual's risk for developing breast cancer. The Royal Melbourne Hospital in Melbourne, Australia, established the Breast and

Ovarian Cancer Risk Management Clinic (BOCRMC) in August 2010 to streamline the assessment and management of high-risk women. The clinic works in collaboration with the Gynaecological Oncology Service at the Royal Women's Hospital, Melbourne, to offer preventive surgery for ovarian cancer. Initially, the BOCRMC referral criteria aligned with the category three FRA-BOC classification as proposed by Cancer Australia and/or eligibility for Medicare coverage of surveillance MRI. However, since late 2015, referral criteria have been defined as a lifetime risk of developing breast cancer of at least 25% based on the BOADICEA and/or eligibility for MRI as per category three of the FRA-BOC. The clinic provides regular breast screening and clinical breast examinations, and advises patients on risk-reducing strategies such as BPM, BPSO, endocrine preventive strategies, and lifestyle recommendations (66).

2 Methodology

2.1 Study 1

The first study represents a case-control study of *BRCA1* and *BRCA2* mutation carriers to evaluate the association between physical activity during adolescence and young adulthood and subsequent risk of breast cancer. The sample comprised baseline and follow-up data from subjects enrolled in a longitudinal study of *BRCA* mutation carriers from 80 participating study centres in 17 countries. Participants were followed prospectively for up to 22 years, accumulating data on reproductive, hormonal and lifestyle factors in *BRCA1/2* mutation carriers. For this study, we included women who were enrolled before 1 August 2017. Case subjects included women with a history of breast cancer. Control subjects were a sample of *BRCA1/2* mutation carriers without a cancer diagnosis and who had not undergone BPM; they were matched on the case subjects on year of birth (± 3 years), place of residence (North America and Poland) and *BRCA* mutation type (*BRCA1* or *BRCA2*). The final sample for analysis were 443 matched sets. All participants completed a detailed questionnaire on family history, personal medical history, as well as various reproductive, hormonal and lifestyle factors, including physical activity. Moderate and vigorous physical activities at ages 12-13, ages 14-17, ages 18-22, ages 23-29 and ages 30-34 were determined using the Nurses' Health Study II Physical Activity Questionnaire. We converted hours per week of moderate and vigorous recreational physical activity to total metabolic equivalents of task (MET) per week overall (ages 12–34), during adolescence (ages 12–17) and during early adulthood (ages 18–34). Logistic regression analysis was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) for total,

moderate and strenuous recreational physical activities and breast cancer risk. Analyses were stratified a priori by menopausal status at breast cancer diagnosis (premenopausal or postmenopausal).

2.2 Study 2

The second study represents a retrospective observational analysis of *BRCA1* and *BRCA2* mutation carriers to investigate the role of the biomarker biologically active Adrenomedulin (bio-ADM) for cardiovascular risk assessment. Thus, we investigated plasma bio-ADM levels in *BRCA1/2* mutation carriers with and without breast cancer and their association with traditional cardiovascular risk factors.

All study subjects are enrolled in an ongoing randomized controlled lifestyle intervention trial in women at increased risk for breast and ovarian cancer (LIBRE) (79). Female *BRCA1/2* mutation carriers were included in this multicentre trial that comprises a feasibility study with 68 women (LIBRE-1), and a confirmatory study with 600 women (LIBRE-2), which is ongoing. The present study is based on samples derived from both the LIBRE-1 and LIBRE-2 cohort. Of the 325 participants who had a blood sample available, we excluded those who had a previous history of ovarian cancer or other cancers than breast cancer. After these exclusions, a total of 292 participants were available for the current analysis. None of the participants had an overt CVD.

The LIBRE trial is a two-armed randomized (1:1) controlled multicentre trial conducted in Germany aimed at determining the impact of a structured one-year lifestyle intervention program on adherence to the Mediterranean Diet (MD), cardiorespiratory fitness and body mass index (BMI) among *BRCA1/2* mutation carriers. The study cohort includes both women with a previous diagnosis of early-stage cancer in remission (diseased) or without a prior cancer diagnosis (non-diseased). The LIBRE-1 study was designed as a feasibility study aimed at determining acceptance and practicability of

the structured intervention program in 68 female mutation carriers. The ongoing LIBRE-2 study is a confirmatory study to examine whether the intervention program is successful in improving the participants' adherence to Mediterranean diet, their physical fitness, but also their quality of life and stress coping capacity. The long-term goals of the intervention program are to provide a reduction of breast cancer incidence, an inhibition of disease progression and a reduction in cancer mortality among *BRCA1/2* mutation carriers.

Bio-ADM was measured using an immunoassay provided by Sphingotec GmbH, Hennigsdorf, Germany. The details of the assay have been published elsewhere (80, 81). All participants completed a detailed interview on personal medical history, as well as various reproductive, hormonal and lifestyle factors. Standardized procedures were used to collect anthropometric measurements, vital signs, cardiorespiratory fitness and nutritional information, including blood tests. Women were categorized into high vs. low plasma bio-ADM based on the median levels in the entire cohort (<13.8 and ≥ 13.8 pg/ml). Logistic regression analysis was performed to estimate the OR and their associated 95% CI of having high circulating bio-ADM levels by different cardiovascular risk factors. A multivariate analysis was carried out to control for potential confounders. These analyses were adjusted for age (years) and history of breast cancer (diseased or non-diseased).

2.3 Study 3

The third study is a retrospective observational analysis of women attending the BOCRMC at the Royal Melbourne Hospital in Melbourne, Australia, to examine the value of a high-risk surveillance program for carriers of an inherited mutation that predisposes to breast cancer and women with strong familial breast cancer risk but without a known gene mutation. The objective of this study was twofold: firstly, to assess the current patient

selection process in the BOCRMC; and secondly, to determine whether the reference criteria should be limited to mutation carriers exclusively or retain a broader scope of women at high risk for breast cancer where no germline mutation was identified. Therefore, we assessed the clinical outcomes of high-risk women attending the BOCRMC with regard to breast malignancies detected through the screening services provided and their uptake rate of preventive options and described these outcomes in both mutation carriers and noncarriers.

A retrospective review was performed using prospectively collected data from women attending the BOCRMC at the Royal Melbourne Hospital between 3 August 2010 and 31 July 2018. A total of 511 women attended the BOCRMC on at least one occasion and were followed-up for up to 96 months. Clinical data from 206 mutation carriers and 305 noncarriers with a ≥25% risk of developing breast cancer were extracted from clinical records and were compared. Demographics, personal and family cancer history, genetic test results, screening history, and risk reduction data were collected. Within the follow-up period of up to 8 years, data included any new cancers detected within the BOCRMC, including detection modality, tumour size, pathology and therapies, updated information on the uptake of risk-reducing services (both risk-reducing surgery and endocrine medical prevention), and if applicable, date and reason for discharge from the clinic.

Due to the small incidence of events, all data were analysed in a descriptive fashion.

3 Publications

3.1 Study 1

Authors: Jacqueline Lammert, Jan Lubinski, Jacek Gronwald, Tomasz Huzarski, Susan Armel, Andrea Eisen, Wendy S. Meschino, Henry T. Lynch, Carrie Snyder, Charis Eng, Olufunmilayo I. Olopade, Ophira Ginsburg, William D. Foulkes, Christine Elser, Stephanie A. Cohen, Marion Kiechle, Steven A. Narod, Joanne Kotsopoulos

Title: Physical activity during adolescence and young adulthood and the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers

Journal: Breast Cancer Research and Treatment

DOI: 10.1007/s10549-018-4694-1

Summary:
Physical activity is known to be inversely related to the risk of breast cancer in the general population. However, it is uncertain whether this association also applies to women with *BRCA*-associated breast cancer.
We conducted a case-control study involving 443 matched pairs of *BRCA* mutation carriers to explore the relationship between physical activity and breast cancer risk. The study assessed moderate and vigorous physical activities during different age periods using the Nurses' Health Study II Physical Activity Questionnaire. The mean metabolic equivalent task hours/week for moderate, vigorous, and total physical activities was estimated for overall (ages 12-34), adolescence (ages 12-17), and early adulthood (ages 18-34). Logistic regression was performed to determine the

OR and 95% CI for total, moderate, and vigorous recreational physical activities and breast cancer risk based on menopausal status.

Overall, there was no significant association between total physical activity and subsequent breast cancer risk ($OR_{Q4\ vs.\ Q1}$ = 1.01, 95% CI 0.69-1.47; *P*-trend = 0.72). However, moderate physical activity between the ages of 12-17 was linked to a 38% lower risk of premenopausal breast cancer ($OR_{Q4\ vs.\ Q1}$ = 0.62; 95% CI 0.40-0.96; *P*-trend = 0.01). There was no association between physical activity and breast cancer diagnosed after menopause.

Early-life physical activity is connected with a decreased risk of premenopausal breast cancer among *BRCA* mutation carriers.

Additional prospective studies combined with mechanistic evidence are necessary to investigate this association further in this high-risk population.

Contribution:

This research project was initiated and successfully completed during a research stay abroad at the Women's College Research Institute, Women's College Hospital in Toronto, Canada. Jacqueline Lammert was accepted for a visiting doctoral student position at Steven Narod's lab from August to September 2017. This research stay was funded by the Internationalization Grant of Technical University of Munich.

Jacqueline Lammert was the principal investigator and author of the published article. She developed the study design, chose the methods to be used and both analysed and interpreted the data with the feedback of Joanne Kotsopoulos and Steven Narod. She wrote the published article, while receiving feedback from her co-authors.

3.2 Study 2

Authors: Jacqueline Lammert, Maryam Basrai, Joachim Struck, Oliver Hartmann, Christoph Engel, Stephan C. Bischoff, Anika Berling-Ernst, Martin Halle, Marion Kiechle, Sabine Grill

Title: Associations of plasma bioactive Adrenomedullin levels with cardiovascular risk factors in *BRCA1/2* mutation carriers

Journal: Geburtshilfe und Frauenheilkunde

DOI: 10.1055/a-1811-2164

Summary:
CVD is a significant cause of morbidity and mortality in breast cancer survivors. However, effective screening methods for identifying CVD risk in this population are currently lacking. ADM has been proposed as a potential biomarker for subclinical cardiac dysfunction in the general population. Lifestyle changes that promote cardiovascular health have been shown to affect ADM levels. Given that *BRCA1/2* mutation carriers have an increased risk of CVD, this study aimed to investigate plasma bio-ADM levels in a cohort of *BRCA* mutation carriers and evaluate their association with cardiovascular risk factors.
Plasma bio-ADM concentrations were measured in 292 female *BRCA1/2* mutation carriers, both with and without a history of breast cancer. Subjects were categorized into high or low bio-ADM levels based on the median bio-ADM level in the entire cohort (13.8 pg/mL). Logistic regression models were utilized to estimate the OR of having elevated bio-ADM levels due to various cardiovascular risk factors.
Among all women in the study (median age: 43 years), 57.5% had a history of breast cancer. The median time between diagnosis and study entry was

three years (range: 0-32 years). Women with metabolic syndrome were found to have a 22-fold increased likelihood of having elevated bio-ADM levels ($P < 0.001$). Elevated bio-ADM levels were associated with lower cardiorespiratory fitness (OR = 0.88, $P < 0.001$) and multiple indicators of obesity ($P < 0.001$). ADM levels were higher in women who had ever smoked (OR = 1.72, $P = 0.02$). However, bio-ADM levels were not associated with a history of breast cancer ($P = 0.28$).
This is the first study that has linked circulating bio-ADM levels to traditional cardiovascular risk factors in *BRCA* mutation carriers. The long-term clinical implications of these findings remain to be determined.

Contribution:
Jacqueline Lammert conceptualized and designed the study, coordinated and conducted the acquisition and interpretation of data, carried out data analyses, and drafted the initial manuscript. This research project was funded by the German Society for Nutritional Medicine (DGEM) and the German Society for Haematology and Medical Oncology (DGHO) within the *DGEM-DGHO-Promotionsstipendium 2018*.

3.3 Study 3

Authors: Jacqueline Lammert, Anita R Skandarajah, Kylie Shackleton, Patricia Calder, Susan Thomas, Geoffrey J. Lindeman, Gregory Bruce Mann
Title: Outcomes of women at high familial risk for breast cancer: An 8-year single-center experience

Journal: Asia Pacific Journal of Clinical Oncology

DOI: 10.1111/ajco.13274

Summary:
The efficacy of a high-risk surveillance program for women at high familial breast cancer risk and mutation carriers has not been thoroughly examined. To provide risk management strategies and multimodality screening, the BOCRMC was established at the Royal Melbourne Hospital in 2010. This study aims to assess the program's effectiveness and describe the incidence of breast cancer diagnoses for *BRCA1*, *BRCA2*, and other germline mutation carriers, as well as high-risk noncarriers attending the BOCRMC.
Clinical data of mutation carriers and noncarriers with a lifetime risk of developing breast cancer ≥25% who attended the clinic between 2010 and 2018 were analysed and described. Cancer detection mode and pattern were determined.
206 mutation carriers and 305 noncarriers underwent screening at the BOCRMC at least once, with a median age of 37 years. After a median follow-up of 34 months, 15 breast cancers (seven invasive) were detected in mutation carriers, and seven (six invasive) in noncarriers. Of these, 20 (90.9%) were detected through annual screening, while two (9.1%) were interval cancers (both in *BRCA1* mutation carriers). The median size of invasive breast cancers was 11 mm (range: 1.5-30 mm), and the majority (76.9%) were axillary node negative. In women aged 25-49 years, the

annualized cancer incidence was 1.6% in *BRCA1*, 1.4% in *BRCA2* mutation carriers, and 0.5% in noncarriers. This is compared to the general Australian population's 0.06% annualized cancer incidence.
Screening proved to be effective at detecting early-stage cancers. The incidence of breast cancer in young noncarriers was significantly higher than the general population. This potentially justifies ongoing management through a specialty clinic, but further research is necessary to personalize risk assessment in noncarriers.

Contribution:
This research project was initiated and successfully completed during a research stay abroad at the Royal Melbourne Hospital, the Peter MacCallum Cancer Centre and the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia. Jacqueline Lammert was accepted for a visiting doctoral student position at Geoffrey Lindeman's lab from March to April 2018. This research stay was funded by the Department of Gynaecology, University Hospital rechts der Isar.
Jacqueline Lammert was the principal investigator and author of the published article. She instigated the research project, developed the study design, collected the clinical data from medical reports, analysed the data and interpreted the data with the feedback of Geoffrey Lindeman and Bruce Mann. She wrote the published article, while receiving feedback from her co-authors.

3.4 Review Article

Authors: Jacqueline Lammert, Sabine Grill, Marion Kiechle

Title: Modifiable Lifestyle Factors: Opportunities for (Hereditary) Breast Cancer Prevention – a Narrative Review

Journal: Breast Care

DOI: 10.1159/000488995

Summary:
Obesity, sedentary behaviour, lack of physical activity, and frequent alcohol consumption are major modifiable risk factors associated with breast cancer. These factors may contribute to approximately one-third of all breast cancer cases. Previous research has primarily focused on assessing the impact of individual risk factors on breast cancer risk while controlling for other confounding variables. However, with the use of big data, efforts are being made to evaluate the combined impact of these well-established lifestyle factors on breast cancer risk. At an individual level, studies suggest that even small changes in lifestyle habits could have a significant impact on breast cancer prevention. Furthermore, emerging research suggests that adopting a healthy lifestyle may be particularly relevant for women with a genetic predisposition to breast cancer. Studies indicate that the presence of certain risk factors, such as excessive body weight, may have a significantly greater impact on breast cancer risk in women with a genetic predisposition to cancer, as compared to the general population. Current research provides guidance for medical professionals to focus on modifiable risk factors when counselling patients. However, randomized controlled trials that utilize objective methods are necessary to provide concrete recommendations for breast cancer prevention.

4 Discussion

The first two studies shed light on modifiable lifestyle factors that contribute to the development of breast cancer and cardiovascular disease in individuals with *BRCA1/2* mutations.

4.1 Association between adolescent physical activity and subsequent breast cancer risk in *BRCA1/2* mutation carriers

The aim of this study was to assess whether physical activity during adolescence and young adulthood is associated with subsequent breast cancer risk among women with a *BRCA1* or *BRCA2* mutation. In general, we did not observe any significant relationship between overall physical activity and breast cancer risk. However, we did find a significant inverse association between increasing levels of moderate-intensity exercise during adolescence and the risk of premenopausal breast cancer (*P*-trend = 0.01). Specifically, women in the highest quartile of physical activity between the ages of 12–17 years had a 38% lower risk of breast cancer (95% CI 0.40–0.96) compared to those in the lowest quartile. We did not find any association between physical activity and breast cancer diagnosed after menopause. Although limited by the retrospective study design, our study is the largest analysis to date conducted specifically among women with a *BRCA1* or *BRCA2* mutation. Taken together, these findings suggest that physical activity during early life may have a significant impact on premenopausal breast cancer risk in this high-risk population (21). King *et al.* were the first to explore the association between adolescent physical activity and subsequent breast cancer risk in *BRCA* mutation carriers (22). The researchers have raised the question whether physical activity might result in a significant delay in the

age of onset of *BRCA*-associated breast cancer. Our research has shown that physical activity conducted during adolescence might be protective against premenopausal cancer, but not postmenopausal cancer. Investigations conducted among the general population suggest that for postmenopausal breast cancer, more recent or sustained physical activity over the course of an individual's lifetime might be important (82, 83). After we published our results of adolescent physical activity, Kehm *et al.* reported on a protective of recreational physical activity in adulthood and subsequent breast cancer risk in a prospective cohort of women at increased risk of breast cancer due to family history, including women with a confirmed germline mutation in *BRCA1* and *BRCA2*; 59% of the sample was premenopausal at baseline (26). Physical activity may affect breast cancer risk through various mechanisms, including metabolic consequences (i.e., hyperinsulinemia, insulin resistance), chronic inflammation, adiposity, elevated levels of growth factors (i.e., insulin-like growth factor 1), as well as an altered sex hormone profile (i.e., oestrogen, progesterone). It is possible that transcriptional changes induced by physical activity mediate these downstream effects, particularly for *BRCA1/2* mutation carriers who are predisposed to genomic instability (21).

When considering lifestyle interventions to prevent *BRCA*-associated breast cancer, it is important to understand that the underlying mechanism of the predisposition is likely different from that of the general population. Haploinsufficiency is a state where a heterozygous individual has only one functional copy of a gene, producing only half the required protein to prevent disease development. It is believed that haploinsufficiency is responsible for the predisposition to breast cancer among *BRCA* mutation carriers since the *BRCA* genes are involved in maintaining genomic integrity through repairing double-stranded DNA breaks. The predisposition to breast cancer among *BRCA* mutation carriers may be due to haploinsufficiency increasing

genomic instability and accelerating the mutation rate of other critical genes, including the second copy of *BRCA1* or *BRCA2*. Therefore, increasing the physiologic expression of the normal copy of the gene and normalizing protein levels may mitigate the effect of the mutation (21). According to a study by Pike *et al.*, the time period between a woman's first period and her first full-term pregnancy is when potential risk factors for developing breast cancer have the most significant impact (84). Our findings support this hypothesis by showing that there is a negative association between physical activity during adolescence and the risk of premenopausal breast cancer, which is consistent with previously published studies. For women with the *BRCA1* mutation, various early-life factors, such as a delayed first period (85), use of oral contraceptives before the age of 25 (86), and breastfeeding for at least one year (87), have been suggested to affect the risk of early-onset breast cancer by influencing the differentiation and proliferation of mammary cells. An in vivo study investigating the association between prepubertal physical activity and alterations in mRNA expression of *BRCA1*, *p53*, oestrogen receptor (ER)-α and ER-β in the mammary glands of adult rats (100 days old) revealed interesting findings. Wang *et al.* found that levels of all four genes, including *BRCA1*, were significantly higher in the mammary glands of exercised rats compared to the sham control rats ($P < 0.03$). Moreover, exercised rats had fewer terminal end buds and a higher number of differentiated alveolar buds and lobules than controls, suggesting fewer targets for neoplastic transformation (88). This study provides valuable insights into possible mechanisms underlying the protective effect of physical activity during childhood or adolescence. There is preliminary evidence to suggest that physical inactivity, namely sedentariness, may alter *BRCA1* gene expression in adult human subjects (89).

For individuals with *BRCA1*-associated breast cancer, the role of progesterone signalling is of particular interest (90). *BRCA* mutation carriers

have higher levels of circulating oestrogen and progesterone (91), which are crucial for mammary gland epithelial cell proliferation and mammary stem cell expansion. Binding of RANKL to RANK in response to progesterone stimulates the proliferation of mammary epithelial cells, whereas osteoprotegerin (OPG) inhibits RANK-associated signal transduction. Women with a *BRCA1* mutation have lower endogenous serum levels of OPG than non-mutation carriers, and inhibiting RANKL has been shown to suppress mammary tumorigenesis in *BRCA1*-deficient mice (92-95). Therefore, inhibiting the progesterone/RANK-signalling pathway may be a promising strategy for the personalized prevention of *BRCA1*-associated breast cancer and is under active study in an ongoing phase three trial (15). This pathway could potentially be influenced by physical activity. Endurance exercise interventions have been shown to decrease luteal phase progesterone levels, and increase circulating OPG while decreasing sRANKL (96, 97). My hypothesis regarding the impact of physical activity on the progesterone/RANK-signalling pathway has sparked a new dissertational project within our research group, see (98). The results of our LIBRE pilot study appear to support my hypothesis, as we observed a rise in OPG and a decline in sRANKL following a 3-month lifestyle intervention. These findings suggest that engaging in more physical activity and following a Mediterranean diet can be regulators of the biomarker OPG, and possibly sRANKL as well (98). Given the prospective nature of the LIBRE trials, we will be able to further elucidate the underlying biological mechanisms of current physical activity on *BRCA*-associated breast cancer risk (99). Findings derived from this study help expand the limited (breast) cancer prevention options currently available to *BRCA1/2* mutation carriers. Ultimately, our goal is to offer data that will support the development of practical and safe lifestyle interventions leading to a decrease in the number of breast cancer cases and deaths attributed to *BRCA1* and *BRCA2* mutations

(100) which provides an attractive adjunct to the currently available prevention strategies.

4.2 Cardiovascular risk assessment in *BRCA1/2* mutation carriers

Mai *et al.* have indicated that individuals with *BRCA1/2* mutations may have a decreased lifespan of about four to six years when compared to the general population, even in the absence of cancer diagnosis (53). While the exact causes of death are unknown, this has spurred investigations into the impact of *BRCA1/2* genes on non-cancerous health conditions such as cardiovascular health.

Apart from cancer recurrence, CVD is the most significant risk of death for women who have had breast cancer due to pre-existing vulnerability and the adverse cardiovascular effects of cancer treatments in the short and long term. Therefore, it is essential to enhance cardiovascular care and manage risk factors in breast cancer patients and survivors to ensure that advancements in cancer treatment do not compromise their cardiovascular health (61).

Breast cancer patients require early detection of subclinical cardiac dysfunction, yet an effective screening program is currently lacking. Various CVD risk scores have been explored, but they are not suitable for this population (61). Echocardiography is widely used, but there is no consensus on follow-up cardiac monitoring. While conventional echocardiography can detect significant structural and functional changes, it may not identify subclinical cardiac impairment until late in the course of CVD. Recent studies suggest that vasoactive peptides, such as ADM, may detect subclinical cardiac impairment earlier than changes in ejection fraction (101).

One approach to making screening more effective is to apply it to a high-risk population, such as *BRCA1/2* mutation carriers, who are suggested to be at an increased risk for CVD. This is the first study that has examined a surrogate marker for CVD, namely bio-ADM, in *BRCA* mutation carriers. High bio-ADM levels were associated with traditional CVD risk factors, including age, body mass index (BMI), insulin resistance, metabolic syndrome, low cardiorespiratory fitness, and smoking. Central obesity was a stronger predictor of high bio-ADM levels than general obesity. There was a significant association between the inflammatory marker high-sensitivity C-reactive protein and bio-ADM. The study found that bio-ADM might be useful in estimating the burden of CVD attributable to modifiable risk factors in *BRCA* mutation carriers (61).

ADM is a peptide that is ubiquitously expressed in the body and has properties that promote vasodilatation and natriuresis. Previous research has found a correlation between high levels of ADM and worse prognosis in patients with heart failure and myocardial infarction. With its superior prognostic value compared to brain natriuretic peptide (102), ADM has a crucial role in the pathophysiology of major cardiac events. Recently, studies conducted on healthy individuals have demonstrated that ADM levels increase years before the onset of CVD and cancer (44, 103). Understanding the mechanisms behind this co-occurrence is of significant public health importance.

While ADM is a well-established biomarker for CVD, its role in breast cancer aetiology is less clear. ADM is expressed in sporadic breast cancer tissue, and its expression is associated with tumour growth, local tumour progression, and bone metastases (104-108). Preliminary evidence suggests that ADM influences the osteoclast differentiation mediated by RANKL (108), an important signalling pathway in *BRCA1*-associated breast carcinogenesis (94, 95).

Based on the robust association between bio-ADM and several risk factors for CVD, our findings indicate that bio-ADM could potentially serve as a

valuable tool in assessing the impact of modifiable risk factors on the development of CVD in individuals with *BRCA* mutations (61).

We relied on bio-ADM measurements taken at baseline only to determine the relationship between ADM and conventional CVD risk factors. Additional investigations are required to establish appropriate bio-ADM thresholds for detecting subclinical cardiac dysfunction. Moreover, the potential long-term clinical outcomes of decreasing bio-ADM levels through lifestyle modifications and/or medical treatments in women with an elevated risk of breast cancer, along with supporting mechanistic evidence, are currently uncertain and warrant further research. As the LIBRE trials are prospective, we will be able to determine how a lifestyle intervention, specifically physical activity and a healthy diet, affects the change in bio-ADM levels over time (61).

4.3 Breast cancer risk management in women at high risk for breast cancer where no germline mutation has been identified

The third study challenges the current approach to patient selection for managing women at high familial risk for breast cancer within a structured breast cancer surveillance program (66).

The main goal of a breast cancer screening program is to detect breast cancers at the earliest stage possible, which is associated with a better prognosis. DCIS is a non-obligate precursor with a very low breast cancer-specific mortality. While the natural history of untreated DCIS is not fully understood, almost all high and intermediate grade lesions and a majority of low-grade lesions will eventually progress to invasive cancer. Therefore, the prevention of breast cancer by diagnosing and treating DCIS is an important objective of a screening program in younger women, particularly as death

from breast cancer after a DCIS diagnosis is substantial for women diagnosed before age 35 (109, 110).

While protocols for managing breast cancer risk in women with *BRCA1/2* mutations are well-established, there is uncertainty regarding the optimal approach for managing the cancer risks of women with a strong family history but no known familial mutation for breast cancer (64).

We evaluated the clinical outcomes of high-risk women who received breast cancer screening services at the BOCRMC, including the detection of breast malignancies and their uptake of preventive options. We described these outcomes for both carriers and noncarriers of mutations (66).

The BOCRMC was established in 2010 to provide risk management strategies and multimodality screening for women at high familial breast cancer risk and mutation carriers, but the efficacy of this program has not been thoroughly examined. Therefore, the aim of this study was to assess the effectiveness of the program and describe the incidence of breast cancer diagnoses for *BRCA1*, *BRCA2*, and other germline mutation carriers, as well as high-risk noncarriers attending the BOCRMC. Clinical data of mutation carriers and noncarriers with a lifetime risk of developing breast cancer ≥25% who attended the clinic between 2010 and 2018 were analysed and compared, with a median age of 37 years for both groups. Of the 206 mutation carriers and 305 noncarriers who underwent screening at the BOCRMC at least once, 15 breast cancers (seven invasive) were detected in mutation carriers, and seven (six invasive) in noncarriers after a median follow-up of 34 months. Among these, 90.9% were detected through annual screening, while two interval cancers (both in *BRCA1* mutation carriers) were detected. The median size of invasive breast cancers was 11 mm (range: 1.5-30 mm), and the majority (76.9%) were axillary node negative. For women aged 25-49 years, the annualized cancer incidence was 1.6% in *BRCA1*, 1.4% in *BRCA2* mutation carriers, and 0.5% in noncarriers,

compared to the general Australian population's 0.06% annualized cancer incidence (66).

While breast cancer risk assessment tools have proven effective when applied to populations, their performance at the individual level and the impact of different thresholds for identifying high-risk individuals is concerning: While comparing various risk prediction models, there is very little consensus in the predicted risk estimates for an individual. However, due to the increasing focus on precision medicine in healthcare, these risk models are now being more widely used to identify women who could potentially benefit from chemoprevention and additional MRI screening (66).

Until 2021, Cancer Australia recommended using category three of the FRA-BOC tool to select patients for intensified breast cancer surveillance in Australia, but although it is easy to use in a clinical setting, it has limitations. In particular, it may underestimate risk in small families with few at-risk female relatives or with paternally inherited risk, and overestimate risk in large families with multiple at-risk female relatives. Additionally, the FRA-BOC online tool does not account for tumour pathology. As an alternative, the BOADICEA risk score has been validated in an Australian population of European ancestry and was introduced to the BOCRMC in 2015. When compared to the FRA-BOC tool, BOADICEA classified only 22.4% of 201 mutation-negative women as high risk using the ≥25% threshold, but 50.7% were eligible when based on a lifetime risk of ≥20% as proposed by the American Cancer Society. Therefore, a significant number of mutation-negative women would not meet inclusion criteria based on BOADICEA alone (66).

BOADICEA was introduced to provide more personalized breast cancer risk assessment, but in our study, six out of seven women without mutations who had screen-detected breast cancers met the FRA-BOC criteria but not the BOADICEA threshold of a lifetime risk of at least 25%. Of these six cases,

four women were diagnosed with breast cancer (3 × IDC, 1 × DCIS) before age 50 years (median age: 38 years, range: 35-48 years). If BOADICEA is likely to underestimate cancer risk in these cases, a combination of validated risk models may be helpful in reducing misclassification bias, as suggested by Park *et al.* (111). To assist with selecting the appropriate breast cancer risk estimation model, the web-based decision support tool iPrevent utilizes initial questions to determine whether IBIS or BOADICEA would be more suitable, thereby providing a more accurate risk assessment at the individual level (112). Mavaddat *et al.* developed a polygenic risk score consisting of 313 SNPs that was optimized for predicting ER-positive and ER-negative disease (PRS313), using the largest available GWAS dataset derived from white European populations (113). This polygenic risk score has the potential to enhance risk stratification for screening and prevention strategies and could be incorporated into risk prediction models, including BOADICEA (114). Further clinical studies are required to evaluate the predictive value of the new PRS313, along with family history and lifestyle risk factors, in the context of current screening protocols. High-risk noncarriers of breast cancer may develop cancers at similar ages as mutation carriers and may require MRI for detection. The multimodal surveillance program implemented at BOCRMC is successful in detecting early-stage breast cancers and the adoption of preventative measures is comparable to that of other studies. Although the FRA-BOC tool provided by Cancer Australia is useful, it may not be optimal in assessing breast cancer risk. BOADICEA was introduced to provide a more accurate estimate of the lifetime risk of breast cancer in noncarriers. However, using a cut-off of 25% lifetime risk on BOADICEA may lead to the dismissal of 77.6% of noncarriers, even though many of these women may be at high risk of developing breast cancer. Moreover, compared to the FRA-BOC tool, BOADICEA may define other women as being at high risk for breast cancer. The incorporation of SNP data and other risk factors may enhance the ability to identify individuals who would benefit the most from intensified surveillance. Further follow-up data is necessary to determine an appropriate

cut-off value for BOADICEA and refine referral criteria for noncarriers in Australia (66).

According to our research, individuals who are not carriers of the *BRCA* gene but have a high risk of developing breast cancer should not be released from the BOCRMC's care. It is crucial to gain a deeper understanding of the genetic factors involved in non-carriers to establish standardized risk assessments and personalized recommendations for breast cancer screening and risk reduction measures. The increasing number of GWAS may offer additional insights into customized risk assessment, optimizing the use of limited resources for maximum benefit (66).

5 References

1. Lord CJ, Ashworth A. BRCAness revisited. Nat Rev Cancer. 2016;16(2):110-20.
2. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA. 2017;317(23):2402-16.
3. Metcalfe K, Lynch HT, Ghadirian P, Tung N, Kim-Sing C, Olopade OI, et al. Risk of ipsilateral breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat. 2011;127(1):287-96.
4. Metcalfe K, Gershman S, Lynch HT, Ghadirian P, Tung N, Kim-Sing C, et al. Predictors of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. Br J Cancer. 2011;104(9):1384-92.
5. Hodgson A, Turashvili G. Pathology of Hereditary Breast and Ovarian Cancer. Front Oncol. 2020;10:531790.
6. Ditsch N, Stickeler E, Behrens A, Belleville E, Fasching PA, Fehm TN, et al. Update Breast Cancer 2021 Part 2 - Advanced Stages, Long-Term Consequences and Biomarkers. Geburtshilfe Frauenheilkd. 2021;81(5):539-48.
7. Woodward ER, Meyer S. Fanconi Anaemia, Childhood Cancer and the BRCA Genes. Genes (Basel). 2021;12(10).
8. Lubinski J, Huzarski T, Byrski T, Lynch HT, Cybulski C, Ghadirian P, et al. The risk of breast cancer in women with a BRCA1 mutation from North America and Poland. Int J Cancer. 2012;131(1):229-34.
9. Tryggvadottir L, Sigvaldason H, Olafsdottir GH, Jonasson JG, Jonsson T, Tulinius H, et al. Population-based study of changing breast cancer risk in Icelandic BRCA2 mutation carriers, 1920-2000. J Natl Cancer Inst. 2006;98(2):116-22.
10. Li N, Rowley SM, Thompson ER, McInerny S, Devereux L, Amarasinghe KC, et al. Evaluating the breast cancer predisposition role of rare variants in genes associated with low-penetrance breast cancer risk SNPs. Breast Cancer Res. 2018;20(1):3.
11. Gaudet MM, Kirchhoff T, Green T, Vijai J, Korn JM, Guiducci C, et al. Common genetic variants and modification of penetrance of BRCA2-associated breast cancer. PLoS Genet. 2010;6(10):e1001183.

12. De Felice F, Marchetti C, Musella A, Palaia I, Perniola G, Musio D, et al. Bilateral risk-reduction mastectomy in BRCA1 and BRCA2 mutation carriers: a meta-analysis. Ann Surg Oncol. 2015;22(9):2876-80.
13. Metcalfe KA, Birenbaum-Carmeli D, Lubinski J, Gronwald J, Lynch H, Moller P, et al. International variation in rates of uptake of preventive options in BRCA1 and BRCA2 mutation carriers. Int J Cancer. 2008;122(9):2017-22.
14. Park SS, Uzelac A, Kotsopoulos J. Delineating the role of osteoprotegerin as a marker of breast cancer risk among women with a BRCA1 mutation. Hered Cancer Clin Pract. 2022;20(1):14.
15. Singer CF. Non-surgical prevention strategies in women with hereditary breast and ovarian cancer syndromes. Horm Mol Biol Clin Investig. 2020;41(3).
16. Kotsopoulos J. BRCA Mutations and Breast Cancer Prevention. Cancers (Basel). 2018;10(12).
17. Liede A, Mansfield CA, Metcalfe KA, Price MA, Kathleen Cuningham Foundation Consortium for Research into Familial Breast C, Snyder C, et al. Preferences for breast cancer risk reduction among BRCA1/BRCA2 mutation carriers: a discrete-choice experiment. Breast Cancer Res Treat. 2017;165(2):433-44.
18. Lammert J, Grill S, Kiechle M. Modifiable Lifestyle Factors: Opportunities for (Hereditary) Breast Cancer Prevention - a Narrative Review. Breast Care (Basel). 2018;13(2):109-14.
19. Islami F, Goding Sauer A, Miller KD, Siegel RL, Fedewa SA, Jacobs EJ, et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. CA Cancer J Clin. 2018;68(1):31-54.
20. Maas P, Barrdahl M, Joshi AD, Auer PL, Gaudet MM, Milne RL, et al. Breast Cancer Risk From Modifiable and Nonmodifiable Risk Factors Among White Women in the United States. JAMA Oncol. 2016;2(10):1295-302.
21. Lammert J, Lubinski J, Gronwald J, Huzarski T, Armel S, Eisen A, et al. Physical activity during adolescence and young adulthood and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat. 2018;169(3):561-71.
22. King MC, Marks JH, Mandell JB, New York Breast Cancer Study G. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003;302(5645):643-6.

23. Nkondjock A, Robidoux A, Paredes Y, Narod SA, Ghadirian P. Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. Breast Cancer Res Treat. 2006;98(3):285-94.
24. Pijpe A, Manders P, Brohet RM, Collee JM, Verhoef S, Vasen HF, et al. Physical activity and the risk of breast cancer in BRCA1/2 mutation carriers. Breast Cancer Res Treat. 2010;120(1):235-44.
25. Grill S, Yahiaoui-Doktor M, Dukatz R, Lammert J, Ullrich M, Engel C, et al. Smoking and physical inactivity increase cancer prevalence in BRCA-1 and BRCA-2 mutation carriers: results from a retrospective observational analysis. Arch Gynecol Obstet. 2017;296(6):1135-44.
26. Kehm RD, Genkinger JM, MacInnis RJ, John EM, Phillips KA, Dite GS, et al. Recreational Physical Activity Is Associated with Reduced Breast Cancer Risk in Adult Women at High Risk for Breast Cancer: A Cohort Study of Women Selected for Familial and Genetic Risk. Cancer Res. 2020;80(1):116-25.
27. Sturgeon KM, Deng L, Bluethmann SM, Zhou S, Trifiletti DM, Jiang C, et al. A population-based study of cardiovascular disease mortality risk in US cancer patients. Eur Heart J. 2019;40(48):3889-97.
28. Blaes AH, Konety SH. Cardiovascular Disease in Breast Cancer Survivors: An Important Topic in Breast Cancer Survivorship. J Natl Cancer Inst. 2021;113(2):105-6.
29. Patnaik JL, Byers T, DiGuiseppi C, Dabelea D, Denberg TD. Cardiovascular disease competes with breast cancer as the leading cause of death for older females diagnosed with breast cancer: a retrospective cohort study. Breast Cancer Res. 2011;13(3):R64.
30. . !!! INVALID CITATION !!! {}.
31. Mehta LS, Watson KE, Barac A, Beckie TM, Bittner V, Cruz-Flores S, et al. Cardiovascular Disease and Breast Cancer: Where These Entities Intersect: A Scientific Statement From the American Heart Association. Circulation. 2018;137(8):e30-e66.
32. Koelwyn GJ, Newman AAC, Afonso MS, van Solingen C, Corr EM, Brown EJ, et al. Myocardial infarction accelerates breast cancer via innate immune reprogramming. Nat Med. 2020;26(9):1452-8.
33. Johnson CB, Davis MK, Law A, Sulpher J. Shared Risk Factors for Cardiovascular Disease and Cancer: Implications for Preventive Health and Clinical Care in Oncology Patients. Can J Cardiol. 2016;32(7):900-7.
34. van Dorst DCH, Dobbin SJH, Neves KB, Herrmann J, Herrmann SM, Versmissen J, et al. Hypertension and Prohypertensive Antineoplastic Therapies in Cancer Patients. Circ Res. 2021;128(7):1040-61.

35. Fraeman KH, Nordstrom BL, Luo W, Landis SH, Shantakumar S. Incidence of new-onset hypertension in cancer patients: a retrospective cohort study. Int J Hypertens. 2013;2013:379252.
36. Gernaat SAM, Ho PJ, Rijnberg N, Emaus MJ, Baak LM, Hartman M, et al. Risk of death from cardiovascular disease following breast cancer: a systematic review. Breast Cancer Res Treat. 2017;164(3):537-55.
37. Gernaat SAM, Boer JMA, van den Bongard DHJ, Maas A, van der Pol CC, Bijlsma RM, et al. The risk of cardiovascular disease following breast cancer by Framingham risk score. Breast Cancer Res Treat. 2018;170(1):119-27.
38. Park NJ, Chang Y, Bender C, Conley Y, Chlebowski RT, van Londen GJ, et al. Cardiovascular disease and mortality after breast cancer in postmenopausal women: Results from the Women's Health Initiative. PLoS One. 2017;12(9):e0184174.
39. Gulati M, Mulvagh SL. The connection between the breast and heart in a woman: Breast cancer and cardiovascular disease. Clin Cardiol. 2018;41(2):253-7.
40. Gernaat SA, Isgum I, de Vos BD, Takx RA, Young-Afat DA, Rijnberg N, et al. Automatic Coronary Artery Calcium Scoring on Radiotherapy Planning CT Scans of Breast Cancer Patients: Reproducibility and Association with Traditional Cardiovascular Risk Factors. PLoS One. 2016;11(12):e0167925.
41. Abdel-Qadir H, Thavendiranathan P, Austin PC, Lee DS, Amir E, Tu JV, et al. Development and validation of a multivariable prediction model for major adverse cardiovascular events after early stage breast cancer: a population-based cohort study. Eur Heart J. 2019;40(48):3913-20.
42. Lyngbakken MN, Myhre PL, Rosjo H, Omland T. Novel biomarkers of cardiovascular disease: Applications in clinical practice. Crit Rev Clin Lab Sci. 2019;56(1):33-60.
43. Funke-Kaiser A, Havulinna AS, Zeller T, Appelbaum S, Jousilahti P, Vartiainen E, et al. Predictive value of midregional pro-adrenomedullin compared to natriuretic peptides for incident cardiovascular disease and heart failure in the population-based FINRISK 1997 cohort. Ann Med. 2014;46(3):155-62.
44. Melander O, Newton-Cheh C, Almgren P, Hedblad B, Berglund G, Engstrom G, et al. Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. JAMA. 2009;302(1):49-57.
45. Kawano S, Kawagoe Y, Kuwasako K, Shimamoto S, Igarashi K, Tokashiki M, et al. Gender-related alterations in plasma adrenomedullin

level and its correlation with body weight gain. Endocr Connect. 2015;4(1):43-9.
46. Vila G, Riedl M, Maier C, Struck J, Morgenthaler NG, Handisurya A, et al. Plasma MR-proADM correlates to BMI and decreases in relation to leptin after gastric bypass surgery. Obesity (Silver Spring). 2009;17(6):1184-8.
47. Ohlsson T, Nilsson PM, Persson M, Melander O. Midregional proadrenomedullin predicts reduced blood pressure and glucose elevation over time despite enhanced progression of obesity markers. J Hypertens. 2019;37(3):590-5.
48. Eggers KM, Venge P, Lindahl B, Lind L. Associations of mid-regional pro-adrenomedullin levels to cardiovascular and metabolic abnormalities, and mortality in an elderly population from the community. Int J Cardiol. 2013;168(4):3537-42.
49. Sajjad M, Fradley M, Sun W, Kim J, Zhao X, Pal T, et al. An Exploratory Study to Determine Whether BRCA1 and BRCA2 Mutation Carriers Have Higher Risk of Cardiac Toxicity. Genes (Basel). 2017;8(2).
50. Schneeweiss A, Hartkopf AD, Muller V, Wockel A, Lux MP, Janni W, et al. Update Breast Cancer 2020 Part 1 - Early Breast Cancer: Consolidation of Knowledge About Known Therapies. Geburtshilfe Frauenheilkd. 2020;80(3):277-87.
51. Gordon T, Kannel WB, Hjortland MC, McNamara PM. Menopause and coronary heart disease. The Framingham Study. Ann Intern Med. 1978;89(2):157-61.
52. Gordhandas S, Norquist BM, Pennington KP, Yung RL, Laya MB, Swisher EM. Hormone replacement therapy after risk reducing salpingo-oophorectomy in patients with BRCA1 or BRCA2 mutations; a systematic review of risks and benefits. Gynecol Oncol. 2019;153(1):192-200.
53. Mai PL, Chatterjee N, Hartge P, Tucker M, Brody L, Struewing JP, et al. Potential excess mortality in BRCA1/2 mutation carriers beyond breast, ovarian, prostate, and pancreatic cancers, and melanoma. PLoS One. 2009;4(3):e4812.
54. Arts-de Jong M, Maas AH, Massuger LF, Hoogerbrugge N, de Hullu JA. BRCA1/2 mutation carriers are potentially at higher cardiovascular risk. Crit Rev Oncol Hematol. 2014;91(2):159-71.
55. Gast KC, Viscuse PV, Nowsheen S, Haddad TC, Mutter RW, Wahner Hendrickson AE, et al. Cardiovascular Concerns in BRCA1 and BRCA2 Mutation Carriers. Curr Treat Options Cardiovasc Med. 2018;20(2):18.

56. van Westerop LL, Arts-de Jong M, Hoogerbrugge N, de Hullu JA, Maas AH. Cardiovascular risk of BRCA1/2 mutation carriers: A review. Maturitas. 2016;91:135-9.
57. Powell CB, Alabaster A, Armstrong MA, Stoller N, Raine-Bennett T. Risk of cardiovascular disease in women with BRCA1 and BRCA2 mutations. Gynecol Oncol. 2018;151(3):489-93.
58. Shukla PC, Singh KK, Quan A, Al-Omran M, Teoh H, Lovren F, et al. BRCA1 is an essential regulator of heart function and survival following myocardial infarction. Nat Commun. 2011;2:593.
59. Singh KK, Shukla PC, Quan A, Al-Omran M, Lovren F, Pan Y, et al. BRCA1 is a novel target to improve endothelial dysfunction and retard atherosclerosis. J Thorac Cardiovasc Surg. 2013;146(4):949-60 e4.
60. Singh KK, Shukla PC, Quan A, Desjardins JF, Lovren F, Pan Y, et al. BRCA2 protein deficiency exaggerates doxorubicin-induced cardiomyocyte apoptosis and cardiac failure. J Biol Chem. 2012;287(9):6604-14.
61. Lammert J, Basrai M, Struck J, Hartmann O, Engel C, Bischoff SC, et al. Associations of Plasma Bioactive Adrenomedullin Levels with Cardiovascular Risk Factors in BRCA1/2 Mutation Carriers. Geburtshilfe Frauenheilkd. 2022;82(6):601-9.
62. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Mol Oncol. 2010;4(3):174-91.
63. Shiovitz S, Korde LA. Genetics of breast cancer: a topic in evolution. Ann Oncol. 2015;26(7):1291-9.
64. Kotsopoulos J, Metcalfe K, Alston J, Nikitina D, Ginsburg O, Eisen A, et al. Prospective study of high-risk, BRCA1/2-mutation negative women: the 'negative study'. BMC Cancer. 2014;14:221.
65. Quante AS, Whittemore AS, Shriver T, Strauch K, Terry MB. Breast cancer risk assessment across the risk continuum: genetic and nongenetic risk factors contributing to differential model performance. Breast Cancer Res. 2012;14(6):R144.
66. Lammert J, Skandarajah AR, Shackleton K, Calder P, Thomas S, Lindeman GJ, et al. Outcomes of women at high familial risk for breast cancer: An 8-year single-center experience. Asia Pac J Clin Oncol. 2020;16(2):e27-e37.
67. Amir E, Freedman OC, Seruga B, Evans DG. Assessing women at high risk of breast cancer: a review of risk assessment models. J Natl Cancer Inst. 2010;102(10):680-91.

68. Harkness EF, Astley SM, Evans DG. Risk-based breast cancer screening strategies in women. Best Pract Res Clin Obstet Gynaecol. 2020;65:3-17.
69. Butler R. Invited Commentary: Breast Cancer Risk Assessment and Screening Strategies-What's New? Radiographics. 2020;40(4):937-40.
70. Ozanne EM, Drohan B, Bosinoff P, Semine A, Jellinek M, Cronin C, et al. Which risk model to use? Clinical implications of the ACS MRI screening guidelines. Cancer Epidemiol Biomarkers Prev. 2013;22(1):146-9.
71. Quante AS, Whittemore AS, Shriver T, Hopper JL, Strauch K, Terry MB. Practical problems with clinical guidelines for breast cancer prevention based on remaining lifetime risk. J Natl Cancer Inst. 2015;107(7).
72. Willoughby A, Andreassen PR, Toland AE. Genetic Testing to Guide Risk-Stratified Screens for Breast Cancer. J Pers Med. 2019;9(1).
73. Zhang Y, Ren H. Meta-analysis of diagnostic accuracy of magnetic resonance imaging and mammography for breast cancer. J Cancer Res Ther. 2017;13(5):862-8.
74. Nelson HD, Smith ME, Griffin JC, Fu R. Use of medications to reduce risk for primary breast cancer: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2013;158(8):604-14.
75. Evans DG, Howell SJ, Howell A. Personalized prevention in high risk individuals: Managing hormones and beyond. Breast. 2018;39:139-47.
76. Mocellin S, Pilati P, Briarava M, Nitti D. Breast Cancer Chemoprevention: A Network Meta-Analysis of Randomized Controlled Trials. J Natl Cancer Inst. 2016;108(2).
77. Evans DG, Wisely J, Clancy T, Lalloo F, Wilson M, Johnson R, et al. Longer term effects of the Angelina Jolie effect: increased risk-reducing mastectomy rates in BRCA carriers and other high-risk women. Breast Cancer Res. 2015;17:143.
78. Evans DG, Baildam AD, Anderson E, Brain A, Shenton A, Vasen HF, et al. Risk reducing mastectomy: outcomes in 10 European centres. J Med Genet. 2009;46(4):254-8.
79. Kiechle M, Engel C, Berling A, Hebestreit K, Bischoff S, Dukatz R, et al. Lifestyle intervention in BRCA1/2 mutation carriers: study protocol for a prospective, randomized, controlled clinical feasibility trial (LIBRE-1 study). Pilot Feasibility Stud. 2016;2:74.
80. Marino R, Struck J, Maisel AS, Magrini L, Bergmann A, Di Somma S. Plasma adrenomedullin is associated with short-term mortality

and vasopressor requirement in patients admitted with sepsis. Crit Care. 2014;18(1):R34.
81. Weber J, Sachse J, Bergmann S, Sparwasser A, Struck J, Bergmann A. Sandwich Immunoassay for Bioactive Plasma Adrenomedullin. J Appl Lab Med. 2017;2(2):222-33.
82. Boeke CE, Eliassen AH, Oh H, Spiegelman D, Willett WC, Tamimi RM. Adolescent physical activity in relation to breast cancer risk. Breast Cancer Res Treat. 2014;145(3):715-24.
83. Liu Y, Tobias DK, Sturgeon KM, Rosner B, Malik V, Cespedes E, et al. Physical activity from menarche to first pregnancy and risk of breast cancer. Int J Cancer. 2016;139(6):1223-30.
84. Pike MC, Krailo MD, Henderson BE, Casagrande JT, Hoel DG. 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. Nature. 1983;303(5920):767-70.
85. Kotsopoulos J, Lubinski J, Lynch HT, Neuhausen SL, Ghadirian P, Isaacs C, et al. Age at menarche and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Cancer Causes Control. 2005;16(6):667-74.
86. Kotsopoulos J, Lubinski J, Moller P, Lynch HT, Singer CF, Eng C, et al. Timing of oral contraceptive use and the risk of breast cancer in BRCA1 mutation carriers. Breast Cancer Res Treat. 2014;143(3):579-86.
87. Kotsopoulos J, Lubinski J, Salmena L, Lynch HT, Kim-Sing C, Foulkes WD, et al. Breastfeeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res. 2012;14(2):R42.
88. Wang M, Yu B, Westerlind K, Strange R, Khan G, Patil D, et al. Prepubertal physical activity up-regulates estrogen receptor beta, BRCA1 and p53 mRNA expression in the rat mammary gland. Breast Cancer Res Treat. 2009;115(1):213-20.
89. Pettapiece-Phillips R, Kotlyar M, Chehade R, Salmena L, Narod SA, Akbari M, et al. Uninterrupted Sedentary Behavior Downregulates BRCA1 Gene Expression. Cancer Prev Res (Phila). 2016;9(1):83-8.
90. Kotsopoulos J, Singer C, Narod SA. Can we prevent BRCA1-associated breast cancer by RANKL inhibition? Breast Cancer Res Treat. 2017;161(1):11-6.
91. Widschwendter M, Rosenthal AN, Philpott S, Rizzuto I, Fraser L, Hayward J, et al. The sex hormone system in carriers of BRCA1/2 mutations: a case-control study. Lancet Oncol. 2013;14(12):1226-32.
92. Atashgaran V, Wrin J, Barry SC, Dasari P, Ingman WV. Dissecting the Biology of Menstrual Cycle-Associated Breast Cancer Risk. Front Oncol. 2016;6:267.

93. Oden L, Akbari M, Zaman T, Singer CF, Sun P, Narod SA, et al. Plasma osteoprotegerin and breast cancer risk in BRCA1 and BRCA2 mutation carriers. Oncotarget. 2016;7(52):86687-94.
94. Nolan E, Vaillant F, Branstetter D, Pal B, Giner G, Whitehead L, et al. RANK ligand as a potential target for breast cancer prevention in BRCA1-mutation carriers. Nat Med. 2016;22(8):933-9.
95. Sigl V, Owusu-Boaitey K, Joshi PA, Kavirayani A, Wirnsberger G, Novatchkova M, et al. RANKL/RANK control Brca1 mutation. Cell Res. 2016;26(7):761-74.
96. Ziegler S, Niessner A, Richter B, Wirth S, Billensteiner E, Woloszczuk W, et al. Endurance running acutely raises plasma osteoprotegerin and lowers plasma receptor activator of nuclear factor kappa B ligand. Metabolism. 2005;54(7):935-8.
97. Bergstrom I, Parini P, Gustafsson SA, Andersson G, Brinck J. Physical training increases osteoprotegerin in postmenopausal women. J Bone Miner Metab. 2012;30(2):202-7.
98. Neirich L, Yahiaoui-Doktor M, Lammert J, Basrai M, Seethaler B, Berling-Ernst A, et al. Physical activity and Mediterranean diet as potential modulators of osteoprotegerin and soluble RANKL in gBRCA1/2 mutation carriers: results of the lifestyle intervention pilot study LIBRE-1. Breast Cancer Res Treat. 2021;190(3):463-75.
99. Kiechle M, Engel C, Berling A, Hebestreit K, Bischoff SC, Dukatz R, et al. Effects of lifestyle intervention in BRCA1/2 mutation carriers on nutrition, BMI, and physical fitness (LIBRE study): study protocol for a randomized controlled trial. Trials. 2016;17:368.
100. Kehm RD, MacInnis RJ, John EM, Liao Y, Kurian AW, Genkinger JM, et al. Recreational Physical Activity and Outcomes After Breast Cancer in Women at High Familial Risk. JNCI Cancer Spectr. 2021;5(6).
101. Pavo N, Raderer M, Hulsmann M, Neuhold S, Adlbrecht C, Strunk G, et al. Cardiovascular biomarkers in patients with cancer and their association with all-cause mortality. Heart. 2015;101(23):1874-80.
102. Yuyun MF, Narayan HK, Ng LL. Prognostic significance of adrenomedullin in patients with heart failure and with myocardial infarction. Am J Cardiol. 2015;115(7):986-91.
103. Daukantaite D, Tellhed U, Maddux RE, Svensson T, Melander O. Five-week yin yoga-based interventions decreased plasma adrenomedullin and increased psychological health in stressed adults: A randomized controlled trial. PLoS One. 2018;13(7):e0200518.

104. Martinez A, Vos M, Guedez L, Kaur G, Chen Z, Garayoa M, et al. The effects of adrenomedullin overexpression in breast tumor cells. J Natl Cancer Inst. 2002;94(16):1226-37.
105. Oehler MK, Fischer DC, Orlowska-Volk M, Herrle F, Kieback DG, Rees MC, et al. Tissue and plasma expression of the angiogenic peptide adrenomedullin in breast cancer. Br J Cancer. 2003;89(10):1927-33.
106. Pare M, Darini CY, Yao X, Chignon-Sicard B, Rekima S, Lachambre S, et al. Breast cancer mammospheres secrete Adrenomedullin to induce lipolysis and browning of adjacent adipocytes. BMC Cancer. 2020;20(1):784.
107. Liu LL, Chen SL, Huang YH, Yang X, Wang CH, He JH, et al. Adrenomedullin inhibits tumor metastasis and is associated with good prognosis in triple-negative breast cancer patients. Am J Transl Res. 2020;12(3):773-86.
108. Awolaran O, Brooks SA, Lavender V. Breast cancer osteomimicry and its role in bone specific metastasis; an integrative, systematic review of preclinical evidence. Breast. 2016;30:156-71.
109. Narod SA, Iqbal J, Giannakeas V, Sopik V, Sun P. Breast Cancer Mortality After a Diagnosis of Ductal Carcinoma In Situ. JAMA Oncol. 2015;1(7):888-96.
110. Husemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, et al. Systemic spread is an early step in breast cancer. Cancer Cell. 2008;13(1):58-68.
111. Park HL, Tran SM, Lee J, Goodman D, Ziogas A, Kelly R, et al. Clinical Implementation of a Breast Cancer Risk Assessment Program in a Multiethnic Patient Population: Which Risk Model to Use? Breast J. 2015;21(5):562-4.
112. Collins IM, Bickerstaffe A, Ranaweera T, Maddumarachchi S, Keogh L, Emery J, et al. iPrevent(R): a tailored, web-based, decision support tool for breast cancer risk assessment and management. Breast Cancer Res Treat. 2016;156(1):171-82.
113. Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, Lee A, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. Am J Hum Genet. 2019;104(1):21-34.
114. Lee A, Mavaddat N, Wilcox AN, Cunningham AP, Carver T, Hartley S, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. Genet Med. 2019;21(8):1708-18.

A Attachments

A.1 List of Publications, Conference Papers & Talks

A.1.1 Research articles as first author

Lammert, J., Basrai, M., Struck, J., Hartmann, O., Engel, C., Bischoff, S. C., Berling-Ernst, A., Halle, M., Kiechle, M., & Grill, S. (2022). Associations of Plasma Bioactive Adrenomedullin Levels with Cardiovascular Risk Factors in *BRCA1/2* Mutation Carriers. *Geburtshilfe und Frauenheilkunde, 82*(6), 601–609. https://doi.org/10.1055/a-1811-2164

Lammert, J., Skandarajah, A. R., Shackleton, K., Calder, P., Thomas, S., Lindeman, G. J., & Mann, G. B. (2020). Outcomes of women at high familial risk for breast cancer: An 8-year single-center experience. *Asia-Pacific Journal of Clinical Oncology*, *16*(2), e27–e37. https://doi.org/10.1111/ajco.13274

Lammert, J., Grill, S., & Kiechle, M. (2018). Modifiable Lifestyle Factors: Opportunities for (Hereditary) Breast Cancer Prevention - a Narrative Review. *Breast Care (Basel, Switzerland)*, *13*(2), 109–114. https://doi.org/10.1159/000488995

Lammert, J., Lubinski, J., Gronwald, J., Huzarski, T., Armel, S., Eisen, A., Meschino, W. S., Lynch, H. T., Snyder, C., Eng, C., Olopade, O. I., Ginsburg, O., Foulkes, W. D., Elser, C., Cohen, S. A., Kiechle, M., Narod, S. A., & Kotsopoulos, J. (2018). Physical activity during adolescence and young adulthood and the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers. *Breast Cancer Research and Treatment*, *169*(3), 561–571. https://doi.org/10.1007/s10549-018-4694-1

A.1.2 Research articles as co-author

Riedel, M., Amann, N., Recker, F., Hennigs, A., Heublein, S., Meyer, B., Karge, A., Eisenkolb, G., Lammert, J., Graf, A., Klein, E., Weiss, M., & Riedel, F. (2022). The COVID-19 pandemic and its impact on medical teaching in obstetrics and gynecology-A nationwide expert survey among teaching coordinators at German university hospitals. *PloS One*, *17*(8), e0269562. https://doi.org/10.1371/journal.pone.0269562

Berling-Ernst, A., Yahiaoui-Doktor, M., Kiechle, M., Engel, C., Lammert, J., Grill, S., Dukatz, R., Rhiem, K., Baumann, F. T., Bischoff, S. C., Erickson, N., Schmidt, T., Niederberger, U., Siniatchkin, M., & Halle, M. (2022). Predictors of cardiopulmonary fitness in cancer-affected and -unaffected women with a pathogenic germline variant in the genes *BRCA1/2* (LIBRE-1). *Scientific Reports*, *12*(1), 2907. https://doi.org/10.1038/s41598-022-06913-1

Grill, S., Yahiaoui-Doktor, M., Basrai, M., Struck, J., Schulte, J., Berling-Ernst, A., Engel, C., Ullrich, M., Lammert, J., Bischoff, S. C., Schmidt, T., Niederberger, U., Chronas, D., Rhiem, K., Schmutzler, R., Halle, M., & Kiechle, M. (2021). Precursor fractions of neurotensin and enkephalin might point to molecular mechanisms of cancer risk modulation during a lifestyle-intervention in germline *BRCA1/2* gene mutation carriers. *Breast Cancer Research and Treatment*, *186*(3), 741–752. https://doi.org/10.1007/s10549-020-06070-x

Neirich, L., Yahiaoui-Doktor, M., Lammert, J., Basrai, M., Seethaler, B., Berling-Ernst, A., Ramser, J., Quante, A. S., Schmidt, T., Niederberger, U., Rhiem, K., Schmutzler, R., Engel, C., Bischoff, S. C., Halle, M., Kiechle, M., & Grill, S. (2021). Physical activity and Mediterranean diet as potential modulators of osteoprotegerin and soluble RANKL in g*BRCA1/2* mutation carriers: results of the lifestyle intervention pilot study LIBRE-1. *Breast Cancer Research and Treatment*, *190*(3), 463–475. https://doi.org/10.1007/s10549-021-06400-7

Pecks, U., Kuschel, B., Mense, L., Oppelt, P., Rüdiger, M., & CRONOS Network (2020). Pregnancy and SARS-CoV-2 Infection in Germany-the CRONOS Registry. *Deutsches Ärzteblatt International*, *117*(49), 841–842. https://doi.org/10.3238/arztebl.2020.0841

Grill, S., Yahiaoui-Doktor, M., Dukatz, R., Lammert, J., Ullrich, M., Engel, C., Pfeifer, K., Basrai, M., Siniatchkin, M., Schmidt, T., Weisser, B., Rhiem, K., Ditsch, N., Schmutzler, R., Bischoff, S. C., Halle, M., & Kiechle, M. (2017). Smoking and physical inactivity increase cancer prevalence in BRCA-1 and BRCA-2 mutation carriers: results from a retrospective observational analysis. *Archives of Gynecology and Obstetrics*, *296*(6), 1135–1144. https://doi.org/10.1007/s00404-017-4546-y

Kiechle, M., Dukatz, R., Yahiaoui-Doktor, M., Berling, A., Basrai, M., Staiger, V., Niederberger, U., Marter, N., Lammert, J., Grill, S., Pfeifer, K., Rhiem, K., Schmutzler, R. K., Laudes, M., Siniatchkin, M., Halle, M., Bischoff, S. C., & Engel, C. (2017). Feasibility of structured endurance training and Mediterranean diet in *BRCA1* and *BRCA2* mutation carriers - an interventional randomized controlled multicenter trial (LIBRE-1). *BMC Cancer*, *17*(1), 752. https://doi.org/10.1186/s12885-017-3732-4

Bertelshofer, F., Brehm, O., Fitzner, P., Lammert, J., Paulsen, F., Janka, R., & Greiner, G. (2016). Skully. In: Tolxdorff, T., Deserno, T., Handels, H., Meinzer, HP. (eds) Bildverarbeitung für die Medizin 2016. Informatik aktuell. Springer Vieweg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-49465-3_44

A.1.3 Conference Talks & Posters

Lammert J (2022). Auswirkung von Ernährung und Sport: Erkenntnisse der LIBRE-Studie auf die Prognose bei Patientinnen mit *BRCA1/2*-Mutation. *41. Jahrestagung der Deutschen Gesellschaft für Senologie e.V. Gemeinsam gegen Brustkrebs: optimale Behandlung für jede Patientin*, Stuttgart, Deutschland.

Lammert J, Grill S, Yahiaoui-Doktor M, Basrai M, Struck J, *et al.* (2020). High circulating levels of adrenomedullin are associated with metabolic syndrome, insulin resistance and low cardiorespiratory fitness in *BRCA1* and *BRCA2* mutation carriers. *Kongress Ernährung 2020 – Medizin fürs Leben*. Virtueller Kongress.
Aktuelle Ernährungsmedizin 2020; 45(03): 228;
DOI: 10.1055/s-0040-1710236.

Lammert J, Grill S, Yahiaoui-Doktor M, Basrai M, Struck J, *et al.* (2020). Abstract P6-10-16: High circulating levels of adrenomedullin are associated with metabolic syndrome and low cardiorespiratory fitness in *BRCA1* and *BRCA2* mutation carriers. *2019 San Antonio Breast Cancer Symposium*.
Cancer Research 80(4_Supplement); December 2019;
DOI: 10.1158/1538-7445.SABCS19-P6-10-16

Lammert J, Grill S, Yahiaoui-Doktor M, Basrai M, Struck J, *et al*. (2019). High circulating levels of adrenomedullin are associated with metabolic syndrome and low cardiorespiratory fitness in *BRCA1* and *BRCA2* mutation carriers. *Jahrestagung der Deutschen, Österreichischen und Schweizerischen Gesellschaften für Hämatologie und Medizinische Onkologie*, Berlin, Germany.

Lammert J (2019). Assessment and management of women at high familial breast cancer risk: from guidelines to clinical practice. *VI. spoločná konferencia SGPS SLS a ČGPS ČLS JEP (6th Joint Conference of*

the Slovak Society of Gynecology and Obstetrics and Czech Society of Gynecology and Obstetrics), Bratislava, Slovakia.

Lammert J, Skandarajah AJ, Shackleton K, Calder P, Susan T, *et al.* (2019). Outcomes of women at high familial risk for breast cancer in Australia: an 8-year single-centre experience. *Royal Australasian College of Surgeons (RACS) 88th Annual Scientific Congress*, Bangkok, Thailand.
ANZ Journal of Surgery (2019) Volume 89, Issue S1 Supplement: Royal Australasian College of Surgeons 88th Annual Scientific Congress, Bangkok, Thailand, 6–10 May 2019. 10.1111/ans.15182

Lammert J (2019). Preventing breast cancer – when the odds are not in your favour. *TEDxBasel 2019*, Basel, Switzerland.

Lammert J, Skandarajah AJ, Shackleton K, Calder P, Susan T, *et al.* (2019). Outcomes of women at high familial risk for breast cancer in Australia: an 8-year single-centre experience. *European Society for Medical Oncology (ESMO) Breast Cancer Annual Congress*, Berlin, Germany.
Annals of Oncology (2019) 30 (suppl_3): iii1-iii26. 10.1093/annonc/mdz095

Lammert J (2019). Die Projektidee BRCA-Mutation.de – Entwicklung und Pilotierung einer evidenzbasierten Informations-Plattform für *BRCA1/2*-Mutationsträgerinnen. *EbM und Digitale Transformation in der Medizin. 20. Jahrestagung des Deutschen Netzwerks Evidenzbasierte Medizin e. V.*, Berlin, Germany.
German Medical Science GMS Publishing House (2019) Doc19ebmP-EG03-07. 10.3205/19ebm062

Neirich L, Kehrer M, Halle M, Berling-Ernst A, Yahiaoui-Doktor M, Bischoff SC, Siniatchkin M, **Lammert J**, Ullrich M, Pfeifer K, *et al.* (2019). LIBRE: Lifestyle Intervention Study in *BRCA1/2* Mutation

Carriers. *International Symposium and Workshop on Physical Activity and Prevention*, Raitenhaslach, Germany.

Lammert J (2018). Preisverleihung Deutsche Gesellschaft für Ernährungsmedizin e.V. (DGEM)-Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie e.V. (DGHO)-Promotionsstipendium 2018: Analyse der risikomodifizierenden Faktoren für die Entstehung des Mammakarzinoms und kardiovaskulären Erkrankungen bei *BRCA1*- und *BRCA2*-Mutationsträgerinnen in der LIBRE-Kohorte. *Ernährung 2018: Ernährung ist Therapie und Prävention*, Kassel, Germany.

Lammert J (2018). TUM-GS INTERNATIONALISIERUNG: Forschungsaufenthalt im Rahmen der Promotion zum Dr. med. *Medizinstudium weltweit*, Munich, Germany.

Lammert J, Lubinski J, Kim SJ, Armel S, Eisen A, *et al.* (2017). Physical activity during adolescence and young adulthood and the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers. *20th International Meeting of the European Society of Gynaecological Oncology (ESGO 2017)*, Vienna, Austria.
International Journal of Gynecological Cancer (2017) Volume 27, Suppl 4: 20th International Meeting of the European Society of Gynaecological Oncology. 10.1097/01.IGC.0000527296.86225.87

Ullrich M, **Lammert J**, Dukatz R, Berling-Ernst A, Yahiaoui-Doktor M, *et al.* (2017). LIBRE: Lebensstil-Interventionstudie bei *BRCA1/2*-Mutationsträgerinnen. *4. Internationaler Sport- und Krebs-Kongress 2017*, Munich, Germany.

Grill S, Yahiaoui-Doktor M, Dukatz R, **Lammert J**, Ullrich M, *et al.* (2017). Smoking and physical inactivity increase cancer prevalence in BRCA-1 and BRCA-2 mutation carriers: results from a retrospective observational analysis. *37. Jahrestagung Deutsche Gesellschaft für Senologie*, Berlin, Germany.

Senologie - Zeitschrift für Mammadiagnostik und -therapie (2017) 14(02): A1-A53
10.1055/s-0037-1602462

Lammert J (2017). Is preventing inherited *BRCA* cancer within our reach? *IV. spoločná konferencia SGPS SLS a ČGPS ČLS JEP (4th Joint Conference of the Slovak Society of Gynecology and Obstetrics and Czech Society of Gynecology and Obstetrics)*, Bratislava, Slovakia.

A.1.4 Other Talks

19.11.2022 **Hirnmetastasen beim HER2-positiven Mammakarzinom: Neue Aspekte bei Diagnostik und Therapie**
FACTUM 2022 - Facts und Cases aus der gynäkologischen Onkologie, Comprehensive Cancer Center Munich, Munich, Germany

30.09.2022 **Highlights vom ESMO-Kongress 2022 – Karzinome der Frau: Ovarialkarzinom**
Comprehensive Cancer Center Munich, Munich, Germany

29.09.2022 **Mammakarzinom: Molekulares Tumorboard – Therapieansätze**
2. Bundeskongress 2022 des Bundesverbands der Study Nurses/Studienassistenten in der klinischen Forschung e.V.
Online Conference

13.11.2021 **Molekulares Tumorboard in der Gynäkoonkologie: Wie kann die personalisierte Onkologie zum Wohle der Patientin in den klinischen Alltag integriert werden?**
FACTUM 2021 - Facts und Cases aus der gynäkologischen Onkologie, Comprehensive Cancer Center Munich, Munich, Germany

19.06.2019 **Workshop: how to identify and avoid predatory journals and conferences**

Mittagsteach, Frauenklinik des Klinikums rechts der Isar der Technischen Universität München, Munich, Germany

27.04.2019 **Preventing breast cancer – when the odds are not in your favour**
TEDxBasel live 2019, Basel, Switzerland

09.11.2018 **Science Pitch** (Winner of the Best Pitch)
Prototype your PhD, UnternehmerTUM, Munich, Germany

04.05.2018 **LIBRE: Lebensstil-Intervention in der Prävention des hereditären Mammakarzinoms**
Frauenklinik des Universitätsklinikums Erlangen, Erlangen, Germany

20.04.2018 **LIBRE study: Is preventing inherited *BRCA* cancer within our reach?**
Parkville Familial Cancer Centre Meeting, Melbourne, Australia

08.10.2017 **LIBRE-Studie: Lebensstil-Intervention in Frauen mit erhöhtem Risiko für Brust- und Eierstockkrebs**
9. Kölner Brustkrebstag, Maritim Hotel Köln, Cologne, Germany

06.10.2017 **Sport in der Prävention von Brustkrebs in *BRCA1/2*-Mutationsträgerinnen**
3. LIBRE-Studientreffen im Rahmen des Kongresses Sport und Krebs 2017, Munich, Germany

16.08.2017 **LIBRE study: Is preventing inherited *BRCA* cancer within our reach?**
Lab Meeting, Women's College Research Institute, Familial Breast Cancer Research Unit
Toronto, Canada

11.07.2017 **LIBRE: Lebensstil-Intervention in der Prävention des hereditären Mammakarzinoms**
MeMPE (Medicine, Master of Public Health and Epidemiology) Summer University 2017 - ein interprofessionelles und praxisorientiertes Seminar

zur Prävention und Gesundheitsförderung
Munich, Germany

30.06.2017 **LIBRE-1 - FEASIBILITY-Studie: Umsetzbarkeit eines strukturierten Ernährungs- und Sportprogramms in *BRCA1/2*-Mutationsträgerinnen**
2. LIBRE-Studientreffen im Rahmen der 37. DGS-Jahrestagung, Berlin, Germany

30.06.2017 **Health status determines motivation towards preventive lifestyle changes in *BRCA1* and *BRCA2* mutation carriers**
2. LIBRE-Studientreffen im Rahmen der 37. DGS-Jahrestagung, Berlin, Germany

28.06.2017 **LIBRE study: Is preventing inherited *BRCA* cancer within our reach?**
Mittagsteach, Frauenklinik des Klinikums rechts der Isar der Technischen Universität München, Munich, Germany

17.08.2016 **LIBRE: Lebensstil-Intervention in der Prävention des hereditären Mammakarzinoms**
Zentrum Familiärer Brust- und Eierstockkrebs, Uniklinik Köln, Cologne, Germany

25.07.2016 **LIBRE: Lebensstil-Intervention in der Prävention des hereditären Mammakarzinoms**
Zertifiziertes interdisziplinäres Brustzentrum am Diakonie Klinikum, Stuttgart, Germany

11.04.2016 **LIBRE: Lebensstil-Intervention in der Prävention des hereditären Mammakarzinoms**
Klinik für Gynäkologie und Geburtshilfe der Universitätsmedizin Göttingen, Göttingen, Germany

Breast Cancer Research and Treatment
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EPIDEMIOLOGY

Physical activity during adolescence and young adulthood and the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers

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Abstract

Background Physical activity is inversely associated with the risk of breast cancer among women in the general population. It is not clear whether or not physical activity is associated with the risk of *BRCA*-associated breast cancer.

Methods We conducted a case–control study of 443 matched pairs of *BRCA* mutation carriers to evaluate the association between physical activity and breast cancer risk. Moderate and vigorous physical activities at ages 12–13, ages 14–17, ages 18–22, ages 23–29 and ages 30–34 were determined using the Nurses' Health Study II Physical Activity Questionnaire. We estimated mean metabolic equivalent task hours/week for moderate, vigorous and total physical activities overall (ages 12–34), during adolescence (ages 12–17) and during early adulthood (ages 18–34). Logistic regression analysis was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) for total, moderate and strenuous recreational physical activities and breast cancer risk, by menopausal status.

Results Overall, there was no significant association between total physical activity and subsequent breast cancer risk ($OR_{Q4\ vs.\ Q1} = 1.01$, 95% CI 0.69–1.47; *P*-trend = 0.72). Moderate physical activity between ages 12–17 was associated with a 38% decreased risk of premenopausal breast cancer ($OR_{Q4\ vs.\ Q1} = 0.62$; 95% CI 0.40–0.96; *P*-trend = 0.01). We found no association between exercise and breast cancer diagnosed after menopause.

Conclusions These findings suggest that early-life physical activity is associated with a reduced risk of premenopausal breast cancer among *BRCA* mutation carriers.

Impact Future prospective analyses, complemented by mechanistic evidence, are warranted in this high-risk population.

Keywords Physical activity · *BRCA1* · *BRCA2* · Breast cancer · Exercise

Introduction

Women who inherit a deleterious *BRCA1* or *BRCA2* mutation face high lifetime risks of developing breast cancer, between 69 and 72% compared to 12% in the general population [1, 2]. Among *BRCA* mutation carriers, primary prevention of breast cancer is limited to prophylactic bilateral mastectomy; however, the majority of women opt for yearly screening with breast magnetic resonance imaging for early detection and frequently inquire about less-invasive prevention options [3, 4]. Although various reproductive and hormonal factors have been shown to influence *BRCA*-associated cancer risk [5], the impact of modifiable lifestyle factors, including physical activity, is not clear.

The relationship between physical activity and breast cancer has been studied extensively among women in the general population. Collectively, the evidence supports an inverse association between physical activity and sporadic breast cancer, with a risk reduction of 25–30% comparing the most physically active to the least active women [6–8]. The relationship exists for both pre- and postmenopausal women, with greater risk reductions reported among postmenopausal women [9]. In contrast, very few studies have evaluated the relationship between physical activity and breast cancer risk specifically among women with a *BRCA1* or *BRCA2* mutation [10]. Although retrospective in nature

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and based on small sample sizes, these studies support a protective role of physical activity during adolescence and early adulthood on subsequent *BRCA*-breast cancer risk [11–13]. In the earliest study, King et al. showed that physical activity during adolescence was associated with a significant delay in the age at the onset of *BRCA*-associated breast cancer [11].

The incomplete penetrance of an inherited *BRCA1* or *BRCA2* mutation suggests that non-genetic factors may influence risk. Furthermore, it is important to integrate evidence-based prevention strategies for the management of this high-risk population. Thus, we conducted a matched, case–control study of 886 *BRCA* mutation carriers to evaluate the association between physical activity in adolescence and in young adulthood and subsequent breast cancer risk.

Subjects and methods

Study population

Eligible study subjects included women enrolled in a longitudinal study of *BRCA1* and *BRCA2* mutation carriers from 80 participating centers in 17 countries. These women sought genetic testing for *BRCA1* and *BRCA2* mutations because of a personal and/or family history of breast and/or ovarian cancer. All study subjects, with the exception of those from the University of Utah and the University of California Irvine, received genetic counseling. Mutation detection was performed using a range of techniques, but all nucleotide sequences were confirmed by direct sequencing of DNA. A woman was eligible for the current study when the molecular analysis established that she was a carrier of a deleterious mutation in the *BRCA1* or *BRCA2* gene. The study was approved by the institutional ethics review boards of the host institutions, and all study subjects provided written informed consent.

Data collection

All study subjects completed a baseline questionnaire at the time of a clinic appointment or at home at a later date. The questionnaire requested detailed information on family history and personal medical histories, as well as various reproductive, hormonal and lifestyle factors. Information on these factors and on incident cancers was updated biennially by follow-up questionnaires. These were either mailed to each study participant to complete and return or were completed over the phone by a genetic counselor or research assistant. In 2014, the Nurses' Health Study II Physical Activity Questionnaire was incorporated into the baseline questionnaire for new participants or into the follow-up questionnaire for women already enrolled in the study [14]. This supplemental questionnaire queried recreational physical activity from ages 12–34 as described in detail below.

Assessment of physical activity

Participants were asked to recall the average duration (0 to $\geq$ 11 h weekly) they engaged in various activities during predefined age periods. Specifically, the questionnaire asked about: (1) moderate recreational activity (e.g., hiking, walking for exercise, casual cycling), and (2) strenuous recreational activity causing increased breathing, heart rate or sweating (e.g., running, aerobics or lap swimming) in grades 7–8 (ages 12–13) and grades 9–12 (ages 14–17) in school and at ages 18–22, ages 23–29 and ages 30–34. Using this information, we computed each participants' metabolic equivalent (MET)-hour/week for each recreational activity type (moderate intensity and high intensity) separately by multiplying duration (average number of hours per week) with the corresponding MET score. In accordance with the Compendium of Physical Activity by Ainsworth [15] and the Nurses' Health Study II [16], we assigned a MET score of 7 to strenuous physical activity and 4.5 to moderate physical activity, respectively. This was estimated for each age category separately and then summed and averaged to estimate the average moderate or strenuous physical activity for overall early-life activity (ages 12–34), adolescent activity (ages 12–17) and young adulthood (ages 18–34). Total recreational physical activity was defined as moderate and vigorous recreational physical activity combined. The total amount of recreational physical activity was assessed by calculating the sum of moderate recreational and vigorous recreational physical activities for each age category separately and then summed and averaged to estimate the average overall physical activity between ages 12–34, plus adolescent (ages 12–17) and young adulthood (ages 18–34) physical activity.

Study subjects available for analysis

There were a total of 2173 potentially eligible *BRCA* mutation carriers who completed the supplemental questionnaire on physical activity, between the years of 2014 and 2017. Subjects were excluded if they had a previous diagnosis of any type of cancer other than breast cancer ($n = 653$), had undergone a prophylactic mastectomy prior to completion of the physical activity questionnaire ($n = 98$), or were missing *BRCA* mutation type ($n = 9$). After these exclusions, a total of 1413 subjects were available for the analysis. Of the 1413 subjects, 554 women had been diagnosed with invasive breast cancer (potential case subjects), whereas the remaining 859 women had never received a cancer diagnosis (potential control subjects).

A single control subject was selected for each case subject, matched according to mutation in the same *BRCA* gene (*BRCA1* or *BRCA2*), year of birth (± 3 years) and place of residence (North America or Poland). In total, 443 matched sets were identified. An appropriate match could not be located for 111 of the eligible cases because the date of birth of these cases was substantially earlier compared to the pool of remaining controls.

Statistical analysis

A matched case–control analysis was performed to evaluate the association between physical activity and the risk of breast cancer. The distributions of continuous variables between cases and controls were compared using the *Student's t* test or, if not applicable due to unequal variances, the *Welch's t* test. The distributions of categorical variables were compared using the Chi-square test. Total, moderate and strenuous physical activities were categorized into quartiles according to the distribution in the controls, with the lowest quartile used as the reference category. Backward stepwise logistic regression analysis was performed to estimate the odds ratios (ORs) and associated 95% confidence intervals (95% CIs) of moderate and strenuous physical activities and a combination of both defined as total recreational physical activity, for each specific age category, plus overall (i.e., between ages 12–34), adolescence (i.e., ages 12–17) and young adulthood (i.e., ages 18–34). A multivariate analysis was carried out to control for potential confounders. All analyses were adjusted for number of children (0, 1–2 or ≥ 3, with 0 used as the reference category), current BMI (with the highest quartile used as the reference category), oral contraception use (ever vs. never), tobacco consumption (ever vs. never) and oophorectomy (ever vs. never). Analyses were stratified a priori by menopausal status at breast cancer diagnosis (premenopausal or postmenopausal). All analyses were performed using SPSS ver. 24.0 (SPSS, Chicago, IL, USA). All *P* values were based on two-sided tests and were considered significant if $P \leq 0.05$.

Results

Case and control subjects were similar with respect to age, reproductive factors, oral contraceptive use, tobacco consumption, alcohol consumption and body mass index (BMI) at age 18 (Table 1). The average age at breast cancer diagnosis was 42.5 years (SD ± 9.1 years). Breast cancer cases were more likely menopausal at the date of questionnaire (91 vs. 73%, $P < 0.001$) and younger at the onset of menopause than controls (44.2 vs. 46.3 years old; $P < 0.001$). The cause of menopause differed between the breast cancer cases and the controls with cases more likely to have undergone treatment-induced menopause (41 vs. 2%; $P < 0.001$). At the date of the physical activity questionnaire, breast cancer cases had a significantly higher BMI compared to controls (26.1 vs. 25.1 kg/m^2, $P = 0.004$).

The relationship between total recreational physical activity (defined as moderate and vigorous recreational physical activity combined) between the ages of 12 and 34 and breast cancer risk among *BRCA* mutation carriers is summarized in Table 2. Among all women combined, there was no significant association between total physical activity between the ages of 12 and 34 and breast cancer comparing the highest versus lowest quartiles of physical activity ($OR_{Q4\ vs.\ Q1} = 1.01$, 95% CI 0.69–1.47; *P*-trend = 0.72). Similarly, there was no relationship between increasing physical activity during adolescence and breast cancer risk ($OR_{Q4\ vs.\ Q1} = 0.94$, 95% CI 0.65–1.36; *P*-trend = 0.13) or early adulthood ($OR_{Q4\ vs.\ Q1} = 1.07$, 95% CI 0.73–1.57; *P*-trend = 0.17). The associations did not vary by menopausal status (*P*-trend > 0.07).

We next evaluated the relationship between moderate-intensity exercise and breast cancer risk (Table 3). Among all women combined, there was no significant association between moderate-intensity exercise from ages 12 to 34 and breast cancer ($OR_{Q4\ vs.\ Q1} = 0.99$, 95% CI 0.68–1.44, *P*-trend = 0.81), as well as moderate recreational physical activity during adolescence ($OR_{Q4\ vs.\ Q1} = 0.82$, 95% CI 0.56–1.20, *P*-trend = 0.09) or early adulthood ($OR_{Q4\ vs.\ Q1} = 1.03$, 95% CI 0.71–1.49, *P*-trend = 0.35). In the analysis stratified by menopausal status at breast cancer diagnosis, there was no significant association between moderate-intensity exercise from ages 12 to 34 and premenopausal breast cancer risk ($OR_{Q4\ vs.\ Q1} = 0.94$, 95% CI 0.62–1.41, *P*-trend = 0.55). Moderate physical activity during adolescence (between ages 12 and 17) was associated with a 38% risk reduction for premenopausal breast cancer ($OR_{Q4\ vs.\ Q1} = 0.62$, 95% CI 0.40-0.96; *P*-trend = 0.01). Compared to a BMI of > 27.98 kg/m^2, a BMI of < 21.28 kg/m^2 was associated with a 42% decreased risk for premenopausal breast cancer ($OR_{Q4\ vs.\ Q1} = 0.58$; 95% CI 0.37–0.93; *P*-trend = 0.047) in the multivariate logistic regression model. Moderate recreational physical activity during early adulthood (between ages 18 and 34) was not associated with premenopausal breast cancer risk ($OR_{Q4\ vs.\ Q1} = 1.03$, 95% CI 0.71–1.49; *P*-trend = 0.12). In the analysis subdivided by age categories, we observed a 45% decreased risk of developing breast cancer before menopause among women in the highest quartile of moderate physical activity between ages 18 and 22 compared to the least active women ($OR_{Q4\ vs.\ Q1} = 0.55$; 95% CI: 0.35–0.87; *P*-trend = 0.006). There was no significant association between increasing moderate-intensity exercise and postmenopausal breast cancer risk overall (highest vs.

Table 1 Baseline characteristics of breast cancer cases and controls among *BRCA* mutation carriers

Characteristic	Controls ($n = 443$)	Breast cancer cases ($n = 443$)	*P*
BRCA mutation status, *n* (%)			
BRCA1	343 (77.4%)		
BRCA2	100 (22.6%)	Matched	
Age (yrs), mean ± SD	50.9 ± 11.6	51.6 ± 10.9	0.31
Age (yrs) at breast cancer diagnosis, mean ± SD (range)	n/a[1]	42.5 ± 9.1 (19-73)	n/a[1]
Country of residence, *n* (%)			
North America	187 (42.2%)		
Poland	256 (57.8%)	Matched	
Age (yrs) at menarche, mean ± SD	13.2 ± 1.4	13 ± 1.6	0.40
Menopausal status, *n* (%)			
Premenopausal	118 (26.6%)	39 (8.8%)	
Postmenopausal	325 (73.4%)	404 (91.2%)	< 0.001
Age (yrs) at menopause, mean ± SD	46.3 ± 6	44.2 ± 6.2	0.001
Cause of menopause, *n* (%)			
Natural	122 (38.7%)	82 (20.4%)	
Surgical (hysterectomy and/or oophorectomy)	187 (59.4%)	154 (38.3%)	
Other[a]	6 (1.9%)	166 (41.3%)	< 0.001
Age at first full-term pregnancy, mean ± SD	25.9 ± 4.9	25.4 ± 4.6	0.11
Number of children, *n* (%)			
0	67 (15.1%)	81 (18.3%)	
1–2	262 (59.1%)	283 (63.9%)	
≥ 3	114 (25.7%)	79 (17.8%)	0.014
Mean ± SD	1.9 ± 1.3	1.7 ± 1.1	0.003
Oral contraceptive use, ever, *n* (%)	263 (59.5%)	258 (58.4%)	0.73
HRT use, ever, *n* (%)	147 (33.2%)	71 (16%)	< 0.001
Smoking status, ever, *n* (%)	173 (39.1%)	195 (44%)	0.13
Alcohol consumption, ever, *n* (%)	381 (86%)	372 (84%)	0.40
BMI (kg/m^2) at age 18, mean ± SD	20.4 ± 2.7	20.7 ± 2.7	0.13
BMI (kg/m^2), mean ± SD	25.1 ± 5.2	26.1 ± 4.7	0.004
Moderate physical activity, ages 12–34, mean ± SD (range)	19.6 ± 13.1 (0–49.5)	18.1 ± 11.6 (0–49.5)	0.12
Strenuous physical activity, ages 12–34, mean ± SD (range)	26.8 ± 18.9 (0–77)	26.4 ± 18.3 (0–77)	0.79

n/a not applicable

[a]Treatment-related (medication or radiotherapy) or other

lowest quartile; $OR_{Q4\ vs.\ Q1} = 1.14$, 95% CI 0.65–2.00, *P*-trend = 0.91), during adolescence ($OR_{Q4\ vs.\ Q1} = 1.53$, 95% CI 0.67–1.98, *P*-trend = 0.51) or early adulthood ($OR_{Q4\ vs.\ Q1} = 1.15$, 95% CI 0.67–1.98, *P*-trend = 0.45).

There was no relationship between vigorous recreational physical activity and breast cancer risk overall ($OR_{Q4\ vs.\ Q1} = 0.94$, 95% CI 0.66–1.35; *P*-trend = 0.35), during adolescence ($OR_{Q4\ vs.\ Q1} = 1.09$, 95% CI 0.77–1.55; *P*-trend = 0.84) and early adulthood ($OR_{Q4\ vs.\ Q1} = 1.00$, 95% CI 0.71–1.39; *P*-trend = 0.36). The associations did not vary by menopausal status (*P*-trend > 0.08) (data not shown).

Discussion

The goal of this study was to evaluate the relationship between physical activity during adolescence and young adulthood and subsequent breast cancer risk among women with a *BRCA1* or *BRCA2* mutation. Overall, we observed no significant association between total physical activity and breast cancer risk. Of interest is the significant inverse association between increasing levels of moderate-intensity exercise during adolescence and the risk of premenopausal breast cancer (*P*-trend = 0.01). Women in

Table 2 Association between total recreational physical activity (in METh) and breast cancer among *BRCA* mutation carriers

	Q1	Q2	Q3	Q4	*P*-trend
All women					
Overall					
Range (METh/week)	≤ 24.65	> 24.65 and ≤ 40.45	> 40.45 and ≤ 63.05	> 63.05	
Cases/Controls	83/88	99/94	98/88	163/173	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.12 (0.73–1.71)	1.22 (0.80–1.88)	1.01 (0.69–1.47)	0.72
Adolescent					
Range (METh/week)	≤ 24.25	> 24.25 and ≤ 40.25	> 40.25 and ≤ 69.25	> 69.25	
Cases/controls	109/107	79/99	125/97	130/140	
Multivariate OR (95% CI)[a]	Ref. 1.00	0.84 (0.56–1.27)	1.33 (0.9–1.96)	0.94 (0.65–1.36)	0.13
Young adulthood					
Range (METh/week)	≤ 20.33	> 20.33 and ≤ 38.58	> 38.58 and ≤ 63	> 63	
Cases/controls	77/91	103/91	114/92	149/169	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.41 (0.92–2.15)	1.44 (0.95–2.19)	1.07 (0.73–1.57)	0.17
Premenopausal at breast cancer diagnosis					
Overall					
Range (METh/week)	≤ 24.65	> 24.65 and ≤ 40.45	> 40.45 and ≤ 63.05	> 63.05	
Cases/controls	62/88	64/94	73/88	118/173	
Multivariate OR (95% CI)[a]	Ref. 1.00	0.96 (0.60–1.53)	1.22 (0.77–1.93)	0.98 (0.65–1.48)	0.70
Adolescent					
Range (METh/week)	≤ 24.25	> 24.25 and ≤ 40.25	> 40.25 and ≤ 69.25	> 69.25	
Cases/controls	78/107	56/99	95/97	88/140	
Multivariate OR (95% CI)[a]	Ref. 1.00	0.83 (0.53–1.31)	1.4 (0.92–2.12)	0.89 (0.59–1.33)	0.07
Young adulthood					
Range (METh/week)	≤ 20.33	> 20.33 and ≤ 38.58	> 38.58 and ≤ 63	> 63	
Cases/controls	54/91	81/91	77/92	105/169	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.57 (0.99–2.49)	1.41 (0.89–2.24)	1.08 (0.71–1.66)	0.13
Postmenopausal at breast cancer diagnosis					
Overall					
Range (METh/week)	≤ 24.65	> 24.65 and ≤ 40.45	> 40.45 and ≤ 63.05	> 63.05	
Cases/controls	21/88	35/94	25/88	43/173	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.63 (0.86–3.0)	1.32 (0.68–2.58)	1.11 (0.61–2.01)	0.40
Adolescent					
Range (METh/week)	≤ 24.25	> 24.25 and ≤ 40.25	> 40.25 and ≤ 69.25	> 69.25	
Cases/controls	31/107	23/99	30/97	40/140	
Multivariate OR (95% CI)[a]	Ref. 1.00	0.89 (0.48–1.67)	1.18 (0.65–2.14)	1.1 (0.63–1.91)	0.84
Young adulthood					
Range (METh/week)	≤ 20.33	> 20.33 and ≤ 38.58	> 38.58 and ≤ 63	> 63	
Cases/controls	23/91	22/91	37/92	42/169	
Multivariate OR (95% CI)[a]	Ref. 1.00	0.94 (0.48–1.84)	1.55 (0.84–2.87)	0.99 (0.55–1.78)	0.28

[a]Estimate adjusted for number of children (0, 1–2 or ≥ 3, with 0 used as the reference category), current BMI (in quartiles, with the highest quartile used as the reference category), oral contraception use (ever/never), tobacco consumption (ever/never), oophorectomy (ever/never)

the highest quartile of physical activity between the ages of 12–17 had a 38% reduction in breast cancer risk (95% CI 0.40–0.96) compared to women in the lowest quartile. We found no association for breast cancer diagnosed after menopause. Although limited by the retrospective study design, our study represents the largest analysis conducted to date specifically among women with a *BRCA1* or *BRCA2* mutation. Collectively, these findings suggest that early-life physical activity may confer a significant impact on premenopausal breast cancer risk in this high-risk population.

Our findings of an inverse association between adolescent physical activity and *BRCA*-breast cancer are consistent with what has previously been reported in three earlier

Table 3 Association between moderate recreational physical activity (in METh) and breast cancer among *BRCA* mutation carriers

	Q1	Q2	Q3	Q4	*P*-trend
All women					
Overall					
Range (METh/week)	≤ 8.55	> 8.55 and ≤ 15.75	> 15.75 and ≤ 27.56	> 27.56	
Cases/controls	93/96	102/97	98/90	150/160	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.1 (0.73–1.65)	1.17 (0.77–1.78)	0.99 (0.68–1.44)	0.81
Adolescent					
Range (METh/week)	≤ 6.75	> 6.75 and ≤ 15.75	> 15.75 and ≤ 25.88	> 25.88	
Cases/controls	136/140	119/114	79/59	80/104	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.09 (0.76–1.56)	1.48 (0.97–2.27)	0.82 (0.56–1.20)	0.09
Young adulthood					
Range (METh/week)	≤ 6.75	> 6.75 and ≤ 15.75	> 15.75 and ≤ 29.25	> 29.25	
Cases/controls	94/100	119/95	84/94	146/154	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.35 (0.90–2.02)	0.99 (0.65–1.50)	1.03 (0.71–1.49)	0.35
Premenopausal at breast cancer diagnosis					
Overall					
Range (METh/week)	≤ 8.55	> 8.55 and ≤ 15.75	> 15.75 and ≤ 27.56	> 27.56	
Cases/controls	67/96	77/97	72/90	101/160	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.17 (0.75–1.83)	1.22 (0.77–1.92)	0.94 (0.62–1.41)	0.55
Adolescent					
Range (METh/week)	≤ 6.75	> 6.75 and ≤ 15.75	> 15.75 and ≤ 25.88	> 25.88	
Cases/controls	104/140	85/114	60/59	47/104	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.04 (0.70–1.53)	1.48 (0.94–2.32)	0.62 (0.40–0.96)	0.01
Young adulthood					
Range (METh/week)	≤ 6.75	> 6.75 and ≤ 15.75	> 15.75 and ≤ 29.25	> 29.25	
Cases/controls	65/100	92/95	65/94	95/154	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.53 (0.99–2.37)	1.11 (0.7–1.74)	0.99 (0.65–1.49)	0.12
Postmenopausal at breast cancer diagnosis					
Overall					
Range (METh/week)	≤ 8.55	> 8.55 and ≤ 15.75	> 15.75 and ≤ 27.56	> 27.56	
Cases/controls	26/96	25/97	25/90	48/160	
Multivariate OR (95% CI)[a]	Ref. 1.00	0.93 (0.49–1.77)	1.05 (0.55–2.01)	1.14 (0.65–2.00)	0.91
Adolescent					
Range (METh/week)	≤ 6.75	> 6.75 and ≤ 15.75	> 15.75 and ≤ 25.88	> 25.88	
Cases/controls	32/140	34/114	18/59	33/104	
Multivariate OR (95% CI)[a]	Ref. 1.00	1.25 (0.71–2.18)	1.39 (0.71–2.73)	1.53 (0.87–2.71)	0.51
Young adulthood					
Range (METh/week)	≤ 6.75	> 6.75 and ≤ 15.75	> 15.75 and ≤ 29.25	> 29.25	
Cases/controls	29/100	26/95	19/94	50/153	
Multivariate OR (95% CI)[a]	Ref. 1.00	0.94 (0.51–1.74)	0.70 (0.36–1.36)	1.15 (0.67–1.98)	0.45

[a]Estimate adjusted for number of children (0, 1–2 or ≥ 3, with 0 used as the reference category), current BMI (in quartiles, with the highest quartile used as the reference category), oral contraception use (ever/never), tobacco consumption (ever/never), oophorectomy (ever/never)

studies. In a group of 104 *BRCA* mutation carriers, King et al. reported a significant delay in breast cancer onset among women who were physically active as teenagers compared to those who were not ($P = 0.03$) [11]. In a retrospective cohort study ($n = 725$), Pijpe et al. reported a significant 42% reduction in risk (hazard ratio (HR) = 0.58; 95% CI 0.35–0.94; *P*-trend = 0.05) with increasing levels of physical activity prior to, but not after, age 30 (hazard ratio = 1.24; 95% CI 0.70–2.19; *P*-trend = 0.51) [12]. In a retrospective analysis of 68 *BRCA* mutation carriers, Grill et al. have shown a significantly lower cancer prevalence in women indicating a higher physical activity level during adolescence compared to their peers ($P = 0.019$) [13]. In contrast, Nkondjock et al. found no association between

levels of physical activity and breast cancer in a case–control study of 137 *BRCA* mutation carriers (total activity: OR = 1.05; 95% CI 0.42–2.60; *P*-trend = 0.91) [17]. Two ongoing prospective randomized controlled trials are evaluating the impact of physical activity and a Mediterranean diet on breast cancer risk and outcomes among 600 *BRCA* mutation carriers [18, 19]. By conducting a follow-up investigation of 13 years, Kiechle et al. also aim to assess the impact of a healthy lifestyle on breast cancer progression and mortality among *BRCA* mutation carriers [18, 20].

In a meta-analysis of 43 studies conducted among women in the general population, Neilson et al. reported that higher versus lower levels of overall moderate to vigorous recreational activity was associated with a 20% decreased risk of premenopausal breast cancer (pooled relative risk = 0.80; 95% CI 0.74–0.87) [21]. Studies evaluating the association between early-life physical activity and premenopausal breast cancer risk have been inconclusive with some groups shown no relationship and others reporting a significant protective effect of adolescent physical activity and the risk of breast cancer prior to menopause [22–24]. Among 75,669 women in the Nurses' Health Study II, Boeke et al. showed a suggestive significant inverse association between physical activity at ages 14–17 (HR = 0.81; 95% CI 0.69–0.95; *P*-trend = 0.10) and at ages 18–22 (HR = 0.85; 95% CI 0.71–1.02; *P*-trend = 0.06), respectively, and premenopausal breast cancer [24]. In contrast, they found no relationship between early-life physical activity and postmenopausal breast cancer risk [25]. We similarly observed no relationship between adolescent or early adulthood physical activity and postmenopausal breast cancer. Given that *BRCA* mutation carriers typically develop breast cancer at an early age, the number of postmenopausal breast cancer cases in our study was relatively small ($n = 124$), and thus, we were not sufficiently powered in our analysis stratified by menopause. However, other researchers suggest that for postmenopausal breast cancer, more recent or sustained physical activity over the lifespan may be of more importance [22, 25]. Obesity is a key factor associated with postmenopausal breast cancer risk among the general population [26]. Manders et al. [27] suggest that excessive body weight increases the risk of postmenopausal, but not premenopausal breast cancer among *BRCA* mutation carriers. In our analysis, we observed a significant association between overweight, i.e., a current BMI of > 27.98 kg/m^2, and premenopausal breast cancer risk. Even though past body size and current body size are highly correlated, this finding should be interpreted with caution as we did not have information about the participants' body size during adolescence and therefore used the information about current BMI, i.e., at a median age of 51, for our multiple regression models. Nevertheless, it is of interest that the association between early-life physical activity and subsequent breast cancer risk was not attenuated by current body size.

There are numerous mechanisms by which physical activity may influence breast cancer risk; increased physical activity may lower cancer risk by reducing insulin resistance, adiposity and chronic inflammation and by decreasing levels of growth factors and endogenous sex hormones [8, 10, 28, 29]. It is also plausible that physical activity-induced transcriptional changes may be mediating these downstream effects [30, 31]. This is of particular relevance for *BRCA* mutation carriers given that their underlying predisposition is the result of a haploinsufficiency state associated with heterozygosity, thereby increasing genomic instability and accelerating the mutation rate of other critical genes (including *BRCA1*) [32]. Wang et al. have shown that physical activity before puberty onset upregulates the *BRCA1* and *p53* mRNA expression, increases the ER-β:ER-α ratio and promotes morphological differentiation of the rat mammary gland [33]. Jackson et al. implicate that *BRCA1* is an important regulator of metabolic function in skeletal muscle in both mouse and human myotubes [34], suggesting that *BRCA1* gene expression might be altered by regular exercise in human subjects.

Of interest for *BRCA1*-associated breast cancer is the emerging role of progesterone signaling [35]. *BRCA* mutation carriers have higher levels of both circulating estrogen and progesterone compared to non-carriers [36]. Progesterone and growth hormones are crucial in mammary gland epithelial cell proliferation and mammary stem cell expansion; breast mitotic activity is highest during the progesterone-dominant luteal phase of the menstrual cycle [36, 37]. Since mammary stem cells are lacking hormone receptors and only a small fraction of mammary epithelial cells express estrogen and progesterone receptors, the regulation of mammary cells through paracrine signaling is of particular relevance [37]. Binding of RANKL to RANK in response to progesterone stimulates the proliferation of mammary epithelial cells, whereas osteoprotegerin (OPG) is an endogenous decoy receptor for RANKL and thereby inhibits RANK-associated signal transduction [35]. Preliminary evidence has shown that women inheriting a *BRCA1* mutation had significantly lower endogenous serum levels of OPG than non-mutation carriers [38]. Two seminal papers recently demonstrated that inhibiting RANKL significantly suppressed mammary tumorigenesis in *BRCA1*-deficient mice [39, 40].

Taken together, these findings implicate the inhibition of the progesterone/RANK-signaling pathway as a promising strategy for the personalized prevention of *BRCA1*-associated breast cancer. Both factors may not only be targeted by chemoprevention, such as *denosumab*, but could potentially be influenced by physical activity. Interestingly, there is some evidence to suggest that endurance exercise

interventions among premenopausal women were able to decrease luteal phase progesterone levels [41, 42], including high-risk women [43]. Furthermore, preliminary evidence suggests that endurance training may induce a significant increase in circulating OPG [44, 45], while decreasing sRANKL [44].

Researchers have postulated that potential risk modifiers for the development of breast cancer are most influential between menarche and first full-term pregnancy [46]. Our findings of an inverse association between adolescent physical activity and premenopausal breast cancer risk line up with a number of previously published results that support this hypothesis. Among *BRCA1* mutation carriers, early exposures, such as late age at menarche [47], oral contraception use prior to, but not after age 25 [48] and breastfeeding for at least 1 year [49], have been proposed to impact early-onset breast cancer risk, among other mechanisms, by influencing mammary cell differentiation and proliferation. Preliminary findings suggest that the impact of physical activity on *BRCA*-associated cancer might be most beneficial when adopted early in life. The duration and intensity of physical activity we found to be protective are in accordance with the recommendations of physical activity of at least one hour of exercise daily among adolescents [50] and are similar to previous findings for sporadic breast cancer [7, 51]. Studies among the general population have shown that both moderate-intensity exercise and vigorous intensity exercise contribute to significant reductions in breast cancer risk, with a greater decrease in risk for vigorous exercise [52]. In contrast, we did not report any association between strenuous recreational physical activity between ages 12 and 34 and breast cancer risk.

Among young women (i.e., between ages 18 and 30) residing in the USA, walking represents the primary form of physical activity, followed by yard work, calisthenics, cycling and dancing, all of which are considered to be of moderate intensity [53]. The significant risk reduction we observed with moderate-intensity exercise could be attributable to a higher prevalence of moderate recreational physical activity and, most possibly, a lower measurement error as compared to vigorous recreational physical activity. Future prospective studies examining different exercise levels and duration, assessed by objective methods, are necessary to elucidate which type of physical activity provides the greatest breast cancer risk reductions among *BRCA* mutation carriers.

Strengths of the current study include the large number of documented *BRCA* mutation carriers which allowed for a priori stratified analyses by menopausal status. In addition, we collected detailed information on lifetime physical activity by using a standardized questionnaire that inquired about duration of physical activity and distinguished between moderate-intensity exercise and high-intensity exercise. We controlled for potential risk factors for *BRCA*-associated breast cancer in our multivariate models, thus decreasing the influence of confounding although our adjusted and unadjusted results did not differ substantially. Therefore, any additional confounding was likely small.

The main limitation of our study was the use of self-reported physical activity which is prone to misclassification; on average, women recalled their early-life physical activity at a mean age of 51. Despite this, an earlier validation study of this instrument reported good reproducibility of recalled activities after 4 years among women enrolled in the Nurses' Health Study II ($r = 0.64$ for total activity) [54]. Given that genetic testing for *BRCA1* and *BRCA2* mutations is offered after age 18, it is unlikely that we would be able to assess exercise during childhood and adolescence prospectively. Furthermore, by comparing extreme quartiles of physical activity, we aimed to discriminate between women who were highly active and those who were relatively inactive; thus, we expect any misclassification to be non-differential. We treated all outcomes as independent hypotheses and did not account for multiple comparisons, and thus, our results should be interpreted with caution. Finally, we were not sufficiently powered to conduct analyses stratified by *BRCA* mutation type.

In summary, the results from our study suggest a protective role of physical activity during adolescence and the risk of premenopausal breast cancer among *BRCA* mutation carriers. Even though bilateral mastectomy remains the most effective risk reduction option, evidence continues to evolve that even in women at highest risk regular physical activity, maintaining a healthy weight and non-smoking may contribute to a decrease in cancer occurrence, especially if adopted early in life [55]. The prospect of physical activity during adolescence for the purpose of *BRCA*-associated cancer prevention, complemented by mechanistic evidence, warrants further evaluation in *BRCA* family-based prospective studies.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ et al (2017) Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. JAMA 317(23):2402–2416
2. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Altekruse SF et al (2016) SEER cancer statistics review, 1975–2013. National Cancer Institute, Bethesda, MD
3. Metcalfe KA, Birenbaum-Carmeli D, Lubinski J, Gronwald J, Lynch H, Moller P et al (2008) International variation in rates of uptake of preventive options in BRCA1 and BRCA2 mutation carriers. Int J Cancer 122(9):2017–2022
4. Liede A, Mansfield CA, Metcalfe KA, Price MA, Snyder C, Lynch HT et al (2017) Preferences for breast cancer risk reduction among BRCA1/BRCA2 mutation carriers: a discrete-choice experiment. Breast Cancer Res Treat 165(2):433–444
5. Narod SA (2010) BRCA mutations in the management of breast cancer: the state of the art. Nat Rev Clin Oncol. 7(12):702–707
6. Wu Y, Zhang D, Kang S (2013) Physical activity and risk of breast cancer: a meta-analysis of prospective studies. Breast Cancer Res Treat 137(3):869–882
7. Friedenreich CM, Cust AE (2008) Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects. Br J Sports Med 42(8):636–647
8. Lynch BM, Neilson HK, Friedenreich CM (2011) Physical activity and breast cancer prevention. Recent Results Cancer Res 186:13–42
9. Henderson KD, Prescott J, Bernstein L (2010) Physical activity and anthropometric factors. In: Li C, (editor). Breast cancer epidemiology. Springer, New York, pp 137–151
10. Pettapiece-Phillips R, Narod SA, Kotsopoulos J (2015) The role of body size and physical activity on the risk of breast cancer in BRCA mutation carriers. Cancer Causes Control 26(3):333–344
11. King MC, Marks JH, Mandell JB (2003) New York Breast Cancer Study G. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 302(5645):643–646
12. Pijpe A, Manders P, Brohet RM, Collee JM, Verhoef S, Vasen HF et al (2010) Physical activity and the risk of breast cancer in BRCA1/2 mutation carriers. Breast Cancer Res Treat 120(1):235–244
13. Grill S, Yahiaoui-Doktor M, Dukatz R, Lammert J, Ullrich M, Engel C et al (2017) Smoking and physical inactivity increase cancer prevalence in BRCA-1 and BRCA-2 mutation carriers: results from a retrospective observational analysis. Arch Gynecol Obstet 296(6):1135–1144
14. Nurses' Health Study II Questionnaire (1997) http://www.nurseshealthstudy.org/sites/default/files/questionnaires/1997long.pdf)
15. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR Jr, Tudor-Locke C et al (2011) 2011 Compendium of physical activities: a second update of codes and MET values. Med Sci Sports Exerc 43(8):1575–1581
16. Maruti SS, Willett WC, Feskanich D, Rosner B, Colditz GA (2008) A prospective study of age-specific physical activity and premenopausal breast cancer. J Natl Cancer Inst 100(10):728–737
17. Nkondjock A, Robidoux A, Paredes Y, Narod SA, Ghadirian P (2006) Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. Breast Cancer Res Treat 98(3):285–294
18. Kiechle M, Engel C, Berling A, Hebestreit K, Bischoff SC, Dukatz R et al (2016) Effects of lifestyle intervention in BRCA1/2 mutation carriers on nutrition, BMI, and physical fitness (LIBRE study): study protocol for a randomized controlled trial. Trials. 17:368
19. Pasanisi P, Bruno E, Manoukian S, Berrino F (2014) A randomized controlled trial of diet and physical activity in BRCA mutation carriers. Fam Cancer 13(2):181–187
20. Kiechle M, Dukatz R, Yahiaoui-Doktor M, Berling A, Basrai M, Staiger V et al (2017) Feasibility of structured endurance training and Mediterranean diet in BRCA1 and BRCA2 mutation carriers—an interventional randomized controlled multicenter trial (LIBRE-1). BMC Cancer. 17(1):752
21. Neilson HK, Farris MS, Stone CR, Vaska MM, Brenner DR, Friedenreich CM (2017) Moderate-vigorous recreational physical activity and breast cancer risk, stratified by menopause status: a systematic review and meta-analysis. Menopause. 24(3):322–344
22. Liu Y, Tobias DK, Sturgeon KM, Rosner B, Malik V, Cespedes E et al (2016) Physical activity from menarche to first pregnancy and risk of breast cancer. Int J Cancer 139(6):1223–1230
23. Oh H, Boeke CE, Tamimi RM, Smith-Warner SA, Wang M, Willett WC et al (2015) The interaction between early-life body size and physical activity on risk of breast cancer. Int J Cancer 137(3):571–581
24. Kruk J (2010) Intensity and timing in life of recreational physical activity in relation to breast cancer risk among pre- and postmenopausal women. J Sports Sci Med 9(2):311–319
25. Boeke CE, Eliassen AH, Oh H, Spiegelman D, Willett WC, Tamimi RM (2014) Adolescent physical activity in relation to breast cancer risk. Breast Cancer Res Treat 145(3):715–724
26. Kerr J, Anderson C, Lippman SM (2017) Physical activity, sedentary behaviour, diet, and cancer: an update and emerging new evidence. Lancet Oncol 18(8):e457–e471
27. Manders P, Pijpe A, Hooning MJ, Kluijt I, Vasen HF, Hoogerbrugge N (2011) Body weight and risk of breast cancer in BRCA1/2 mutation carriers. Breast Cancer Res Treat 126(1):193–202
28. Himbert C, Delphan M, Scherer D, Bowers LW, Hursting S, Ulrich CM (2017) Signals from the adipose microenvironment and the obesity-cancer link-a systematic review. Cancer Prev Res (Phila). 10(9):494–506
29. Hoffman-Goetz L, Apter D, Demark-Wahnefried W, Goran MI, McTiernan A, Reichman ME (1998) Possible mechanisms mediating an association between physical activity and breast cancer. Cancer 83(3 Suppl):621–628
30. Liu D, Sartor MA, Nader GA, Gutmann L, Treutelaar MK, Pistilli EE et al (2010) Skeletal muscle gene expression in response to resistance exercise: sex specific regulation. BMC Genomics 11:659
31. Radom-Aizik S, Zaldivar F, Jr., Leu SY, Cooper DM (2009) A brief bout of exercise alters gene expression and distinct gene pathways in peripheral blood mononuclear cells of early- and late-pubertal females. J Appl Physiol (1985) 107(1):168–175
32. Salmena L, Narod S (2012) BRCA1 haploinsufficiency: consequences for breast cancer. Womens Health (Lond). 8(2):127–129
33. Wang M, Yu B, Westerlind K, Strange R, Khan G, Patil D et al (2009) Prepubertal physical activity up-regulates estrogen receptor beta, BRCA1 and p53 mRNA expression in the rat mammary gland. Breast Cancer Res Treat 115(1):213–220

34. Jackson KC, Gidlund EK, Norrbom J, Valencia AP, Thomson DM, Schuh RA et al (2014) BRCA1 is a novel regulator of metabolic function in skeletal muscle. J Lipid Res 55(4):668–680
35. Kotsopoulos J, Singer C, Narod SA (2017) Can we prevent BRCA1-associated breast cancer by RANKL inhibition? Breast Cancer Res Treat 161(1):11–16
36. Widschwendter M, Rosenthal AN, Philpott S, Rizzuto I, Fraser L, Hayward J et al (2013) The sex hormone system in carriers of BRCA1/2 mutations: a case-control study. Lancet Oncol. 14(12):1226–1232
37. Atashgaran V, Wrin J, Barry SC, Dasari P, Ingman WV (2016) Dissecting the biology of menstrual cycle-associated breast cancer risk. Front Oncol 6:267
38. Oden L, Akbari M, Zaman T, Singer CF, Sun P, Narod SA et al (2016) Plasma osteoprotegerin and breast cancer risk in BRCA1 and BRCA2 mutation carriers. Oncotarget. 7(52):86687–86694
39. Nolan E, Vaillant F, Branstetter D, Pal B, Giner G, Whitehead L et al (2016) RANK ligand as a potential target for breast cancer prevention in BRCA1-mutation carriers. Nat Med 22(8):933–939
40. Sigl V, Owusu-Boaitey K, Joshi PA, Kavirayani A, Wirnsberger G, Novatchkova M et al (2016) RANKL/RANK control Brca1 mutation-driven mammary tumors. Cell Res 26(7):761–774
41. Ahrens KA, Vladutiu CJ, Mumford SL, Schliep KC, Perkins NJ, Wactawski-Wende J et al (2014) The effect of physical activity across the menstrual cycle on reproductive function. Ann Epidemiol 24(2):127–134
42. El-Lithy A, El-Mazny A, Sabbour A, El-Deeb A (2015) Effect of aerobic exercise on premenstrual symptoms, haematological and hormonal parameters in young women. J Obstet Gynaecol 35(4):389–392
43. Kossman DA, Williams NI, Domchek SM, Kurzer MS, Stopfer JE, Schmitz KH (2011) Exercise lowers estrogen and progesterone levels in premenopausal women at high risk of breast cancer. J Appl Physiol (1985) 111(6):1687–1693
44. Ziegler S, Niessner A, Richter B, Wirth S, Billensteiner E, Woloszczuk W et al (2005) Endurance running acutely raises plasma osteoprotegerin and lowers plasma receptor activator of nuclear factor kappa B ligand. Metabolism. 54(7):935–938
45. Bergstrom I, Parini P, Gustafsson SA, Andersson G, Brinck J (2012) Physical training increases osteoprotegerin in postmenopausal women. J Bone Miner Metab 30(2):202–207
46. Pike MC, Krailo MD, Henderson BE, Casagrande JT, Hoel DG (1983) 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. Nature 303(5920):767–770
47. Kotsopoulos J, Lubinski J, Lynch HT, Neuhausen SL, Ghadirian P, Isaacs C et al (2005) Age at menarche and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Cancer Causes Control 16(6):667–674
48. Kotsopoulos J, Lubinski J, Moller P, Lynch HT, Singer CF, Eng C et al (2014) Timing of oral contraceptive use and the risk of breast cancer in BRCA1 mutation carriers. Breast Cancer Res Treat 143(3):579–586
49. Kotsopoulos J, Lubinski J, Salmena L, Lynch HT, Kim-Sing C, Foulkes WD et al (2012) Breastfeeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res 14(2):R42
50. 2008 Physical activity guidelines for Americans. Okla Nurse 53(4):25
51. Thune I, Brenn T, Lund E, Gaard M (1997) Physical activity and the risk of breast cancer. N Engl J Med 336(18):1269–1275
52. Friedenreich CM (2010) The role of physical activity in breast cancer etiology. Semin Oncol 37(3):297–302
53. Dowda M, Ainsworth BE, Addy CL, Saunders R, Riner W (2003) Correlates of physical activity among U.S. young adults, 18 to 30 years of age, from NHANES III. Ann Behav Med 26(1):15–23
54. Wolf AM, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Corsano KA et al (1994) Reproducibility and validity of a self-administered physical activity questionnaire. Int J Epidemiol 23(5):991–999
55. Terry MB, Bradbury A (2016) Family-based breast cancer prevention efforts in adolescence. Pediatrics 138(Supplement 1):S78–S80

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Associations of Plasma Bioactive Adrenomedullin Levels with Cardiovascular Risk Factors in *BRCA1/2* Mutation Carriers

Zusammenhang zwischen bioaktivem Adrenomedullin-Spiegel und kardiovaskulären Risikofaktoren bei *BRCA1/2*-Mutationsträgerinnen

OPEN ACCESS

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Bibliography

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ABSTRACT

Background Cardiovascular disease (CVD) is an important cause of morbidity and mortality in breast cancer survivors. Effective screening modalities to identify CVD risk are lacking in this population. Adrenomedullin (ADM) has been suggested as a biomarker for subclinical cardiac dysfunction in the general population. Levels of ADM have been proven to be responsive to lifestyle changes that lead to improved cardiovascular health. As *BRCA1/2* mutation carriers are deemed to be at an increased risk for CVD, the aim of this study was to examine plasma ADM levels in a cohort of *BRCA* mutation carriers and to assess their association with cardiovascular risk factors.

Methods Plasma ADM concentrations were measured in 292 female *BRCA1/2* mutation carriers with and without a history of breast cancer. Subjects were classified into high versus low ADM levels based on the median ADM level in the entire cohort (13.8 pg/mL). Logistic regression models were used to estimate the odds ratios (OR) of having elevated ADM levels by several cardiovascular risk factors.

Results Of all women (median age: 43 years), 57.5% had a previous diagnosis of breast cancer. The median time between diagnosis and study entry was three years (range: 0–32 years). Women presenting with metabolic syndrome had 22-fold increased odds of having elevated ADM levels ($p < 0.001$). Elevated ADM levels were associated with lower cardiorespiratory fitness (OR = 0.88, $p < 0.001$) and several parameters of obesity ($p < 0.001$). ADM levels were higher in women who have ever smoked (OR = 1.72, $p = 0.02$). ADM levels were not associated with a previous diagnosis of breast cancer ($p = 0.28$).

Conclusions This is the first study in *BRCA* mutation carriers that has linked circulating ADM levels to traditional cardiovascular risk factors. The long-term clinical implications of these findings are yet to be determined.

ZUSAMMENFASSUNG

Hintergrund Herz-Kreislauf-Erkrankungen (HKE) sind eine wichtige Ursache für Morbidität und Mortalität bei Brustkrebsüberlebenden. Es fehlt aber an effektiven Früherkennungsuntersuchungen, welche die HKE-Risiken in dieser Population identifizieren könnten. Adrenomedullin (ADM) wurde bereits als möglicher Biomarker für subklinische Herzerkrankungen in der Allgemeinbevölkerung vorgeschlagen. Es hat sich gezeigt, dass Lebensstiländerungen, die zu einer Verbesserung der kardiovaskulären Gesundheit führen, sich in ADM-Plasmakonzentrationen widerspiegeln. Da Trägerinnen von *BRCA1/2*-Mutationen ein erhöhtes HKE-Risiko haben, zielt diese Studie darauf ab, die ADM-Plasmakonzentrationen in einer Gruppe von *BRCA*-Mutationsträgerinnen zu messen und den Zusammenhang mit HKE-Risikofaktoren zu untersuchen.

Methoden ADM-Plasmakonzentrationen wurden in 292 *BRCA1/2*-Mutationsträgerinnen mit oder ohne frühere Brustkrebsdiagnose gemessen. Basierend auf der medianen ADM-Konzentration der Gesamtgruppe (13,8 pg/ml) wurden die untersuchten Frauen gemäß ihrer ADM-Konzentrationen in 2 Gruppen (hohe bzw. niedrige ADM-Konzentration) eingeteilt. Logistische Regressionsmodelle wurden verwendet, um das Chancenverhältnis (OR) verschiedener kardiovaskulärer Risikofaktoren in Abhängigkeit der Höhe der ADM-Konzentration zu schätzen.

Ergebnisse Bei 57,5 % der Frauen (Durchschnittsalter: 43 Jahre) wurde zuvor Brustkrebs diagnostiziert. Die mediane Zeit zwischen der Krebsdiagnose und die Aufnahme in dieser Studie betrug 3 Jahre (Spanne: 0–32 Jahre). Frauen mit metabolischem Syndrom hatten eine 22-fach höhere Wahrscheinlichkeit eines erhöhten ADM-Spiegels ($p < 0{,}001$). Erhöhte ADM-Spiegel waren mit niedriger kardiorespiratorischer Fitness (OR = 0,88, $p < 0{,}001$) sowie verschiedenen Übergewichtsparametern ($p < 0{,}001$) assoziiert. Der ADM-Spiegel war höher bei Frauen, die rauchten bzw. früher geraucht hatten (OR = 1,72, $p = 0{,}02$). Es gab kein Zusammenhang zwischen ADM-Konzentrationen und einer früheren Brustkrebsdiagnose ($p = 0{,}28$).

Schlussfolgerungen Dies ist die erste Studie von *BRCA*-Mutationsträgerinnen, welche die Verbindung zwischen ADM-Plasmakonzentrationen und traditionellen kardiovaskulären Risikofaktoren untersucht. Die langfristigen klinischen Implikationen der Befunde müssen noch ermittelt werden.

Introduction

With continual improvements in cancer outcomes, cardiovascular disease (CVD) is an important cause of morbidity and mortality in (early) breast cancer patients [1]. In fact, risk of death from cardiovascular causes surpasses the risk of death from breast cancer eight years after diagnosis [2, 3]. CVD can be caused or accelerated by a variety of breast cancer treatments, including anthracycline chemotherapy, Her2-targeted therapy, chest radiation therapy and long-term oestrogen suppression [4, 5]. Reciprocally, studies in mice and humans have shown that a serious cardiac event, such as a myocardial infarction, accelerates breast cancer outgrowth and cancer-specific mortality [6]. Additionally, there is a significant overlap of risk factors common to both diseases, including aging, physical inactivity and metabolic syndrome [7]. Thus, breast cancer survivors have been shown to have a higher prevalence of cardiovascular risk factors than age-matched, cancer-unaffected women [8, 9].

Secondary prevention of CVD is dependent upon the ability to identify high-risk individuals long before the development of cardiac injury.

Given the long latency periods between the initial diagnosis of breast cancer and manifest CVD of approximately five to seven years [10, 11], there is a window of opportunity to identify and treat CVD risk factors before any clinical signs or symptoms become evident.

One of the barriers to improving cardiovascular disease outcomes in breast cancer survivors is the lack of reliable, effective screening modalities. Traditional risk assessment tools, such as the Framingham Risk Score, significantly underestimate a breast cancer survivor's risk of developing CVD [9, 12], highlighting the importance of specific CVD assessment in these women [13].

An increasing number of biomarkers has been identified to predict cardiovascular events among the general population [14]. The value of blood-based biomarkers to identify preclinical CVD in breast cancer survivors has not yet been evaluated.

Adrenomedullin (ADM) represents one of the candidate markers that predict vascular changes, and it becomes elevated years before the onset of non-communicable diseases [15]. In particular, increased levels of ADM among healthy individuals are strongly associated with later development of CVD and cancer, as well as premature mortality [16]. Moreover, studies suggest that ADM is responsive to lifestyle and metabolic changes that lead to improved cardiovascular health [17 – 20].

It is well established that *BRCA1/2* mutation carriers have a high lifetime risk of developing breast cancer. Having a risk of 69–72 % of developing breast cancer and a risk of 17–44 % for developing ovarian cancer by age 80 years [21], *BRCA1/2* mutation carriers are exposed to cancer treatments and prophylactic bilateral salpingo-oophorectomy (PBSO) with detrimental short- and long-term effects on cardiovascular health [22]. Firstly, women with *BRCA*-associated breast cancers are typically diagnosed before age 50 years [21], which is substantially younger than the median age at breast cancer diagnosis of 64 years in the general population [23]. They also have a high risk of developing contralateral [24] or ipsilateral cancer [25]. Secondly, *BRCA*-associated cancers exhibit pathological features suggestive of an aggressive phenotype (e.g., G3 cancers, basal-like disease in *BRCA1* mutation carriers and luminal B tumours in *BRCA2* mutation carriers) [26, 27], and therefore, most patients undergo potentially cardiotoxic che-

motherapy. Thirdly, when diagnosed with ER-positive breast cancer, patients might benefit from an extended adjuvant endocrine therapy [28]. Additionally, *BRCA1/2* mutation carriers are advised to undergo PBSO after child-bearing age. Long-term oestrogen deprivation in women undergoing PBSO has been shown to increase CVD risk by two- to threefold as compared to women of the same age without surgical menopause [29, 30]. Preliminary evidence indicates that *BRCA1/2* mutation carriers are more prone to cardiovascular disease both at baseline and in response to cancer treatments [31 – 35]. Recent research suggests that the *BRCA* genes regulate cardiomyocyte survival and function, and that loss of function leads to increased susceptibility to cardiac damage [33 – 35]. Experimental findings in mice have demonstrated that *BRCA1* limits endothelial cell apoptosis, restores endothelial function, and attenuates atherosclerotic lesion development [36]. Moreover, loss of *BRCA2* has been shown to increase susceptibility to doxorubicin-induced heart failure [37]. Therefore, a biomarker to determine cardiovascular risk might be of particular relevance to *BRCA1/2* mutation carriers.

In this study, we investigated plasma ADM levels in *BRCA1/2* mutation carriers with and without breast cancer and their association with traditional cardiovascular risk factors.

Methods

Study population

The participants under investigation were enrolled in the randomized controlled LIBRE-2 trial (Lifestyle intervention study in women with hereditary breast and ovarian cancer) and the associated feasibility study LIBRE-1. The trials are registered at ClinicalTrials.gov (NCT numbers: NCT02087592 – registered on 14/03/2014, NCT02516540 – registered on 06/08/2015).

The LIBRE-2 trial is an ongoing, two-armed randomized (1 : 1) controlled multicentre trial conducted in Germany aimed at determining the impact of a structured one-year lifestyle intervention program on adherence to the Mediterranean Diet, cardiorespiratory fitness and BMI among *BRCA1/2* mutation carriers. The study cohort includes both women with a previous diagnosis of early-stage cancer in remission (diseased) or without a prior cancer diagnosis (non-diseased). Details on the study design have been published elsewhere [38, 39].

Of the 325 participants who had a blood sample available, we excluded those who had a previous history of ovarian cancer or other cancers than breast cancer. After these exclusions, a total of 292 participants were available for the current analysis. None of the participants had an overt CVD.

The study was approved by the institutional ethics review boards of both the host institutions (Technical University of Munich: Reference No. 5686/13, University Hospital Cologne: Reference No. 13-053 and University Hospital Schleswig-Holstein in Kiel: Reference No. B-235/13) and the participating study centers, and all study subjects provided written informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Data collection

For this study, all measurements, including biologically active ADM, were captured at baseline.

At baseline, participants completed a standardized questionnaire to collect detailed information on medical history, demographic data as well as various reproductive, hormonal and lifestyle factors. Adherence to the Mediterranean Diet was captured by the Mediterranean Diet Adherence Screener (MEDAS), a validated questionnaire consisting of 14 items [40]. We calculated the MEDAS score ranging from 0 to 14 as a percentage of positively answered questions. At enrolment, all participants underwent physical examination to collect systolic and diastolic blood pressure, resting heart rate and anthropometric measurements (i.e., weight [kg], height [m], waist [cm], and hip circumferences [cm]). The four anthropometric measurements were used to calculate body mass index (kg/m^2) and waist-to-hip-ratio (waist circumference [cm]/hip circumference [cm]).

Specimen collection and analysis

All routine analyses were performed by affiliated laboratories of local institutions. Blood samples were withdrawn after an overnight fast for at least 12 hours for assessment of the serum levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose, insulin and high-sensitivity C-reactive protein (hs-CRP) using standard procedures.

Insulin Resistance (IR) was calculated using the homeostasis model assessment (HOMA-IR) equation formula as follows: HOMA-IR = fasting insulin (µU/mL) multiplied by fasting glucose (mmol/L) divided by 22.5. IR was defined as HOMA-IR ≥ 2.5.

Metabolic syndrome was defined according to the International Diabetes Federation (IDF) criteria by the presence of a waist circumference of ≥ 80 cm together with at least two of the following metabolic abnormalities:

a. fasting blood glucose ≥ 100 mg/dL;
b. systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg;
c. triglycerides ≥ 150 mg/dL;
d. HDL-cholesterol < 50 mg/dL.

The definition also considered treatment with the use of lipid-lowering, glucose-lowering, and antihypertensive drugs.

For blinded ADM analysis, EDTA samples were processed and stored at − 80 °C before transfer to the central laboratory of Sphingotec GmbH. Biologically active Adrenomedullin (bio-ADM) was measured using an immunoassay provided by Sphingotec GmbH, Hennigsdorf, Germany. Details on the assay have been published elsewhere [41, 42]. The analytical assay sensitivity was 2 pg/mL.

Physical activity assessment

Cardiorespiratory fitness was determined by peak oxygen uptake (VO_{2peak}) and assessed via cardiopulmonary exercise testing (CPET). The CPET was a ramp protocol (3 minutes sitting on the bicycle, 3 minutes steady state at 30 watts, continuous individual increase in wattage with the aim of achieving a maximal workload on the test person within 8 to 12 minutes, 5 minutes recovery

▶ **Table 1** Baseline characteristics by median Adrenomedullin levels.

Characteristic	bio-ADM < 13.8 pg/mL (n = 147)	bio-ADM ≥ 13.8 pg/mL (n = 145)
Age, years, mean ± SD	41.2 ± 9.7	43.8 ± 10.4
BRCA mutation status, n (%)		
▪ *BRCA1*	96 (33.2%)	87 (30.1%)
▪ *BRCA2*	46 (15.9%)	56 (19.4%)
▪ *BRCA1* and *BRCA2*	2 (0.7%)	2 (0.7%)
Parous, n (%)	90 (30.8%)	95 (32.5%)
Prophylactic bilateral salpingo-oophorectomy (PBSO), n (%)		
▪ Yes	44 (15.1%)	36 (12.3%)
▪ No	102 (34.9%)	110 (37.7%)
Age at PBSO, years, median (range)	45 (29–60)	45 (36–65)
Breast cancer		
Previous diagnosis of breast cancer, n (%)		
▪ Non-diseased	67 (22.9%)	57 (19.5%)
▪ Diseased	80 (27.4%)	88 (30.1%)
Age at breast cancer diagnosis, years, mean ± SD	39.3 ± 8	40.3 ± 8.3
Time between breast cancer diagnosis and study entry, years, mean ± SD	4.5 ± 6	4.9 ± 5.1
Tumour biology		
▪ Hormone receptor-positive	31 (18.4%)	38 (22.6%)
▪ HER2-positive	3 (1.8%)	3 (1.8%)
▪ Triple negative	46 (27.4%)	47 (28%)
Breast cancer treatments		
▪ Chemotherapy	63 (37.5%)	61 (36.3%)
▪ Chest radiation therapy	42 (25%)	46 (27.4%)
▪ Antihormonal treatment	31 (18.4%)	38 (22.6%)
▪ HER2-targeted treatment	3 (1.8%)	3 (1.8%)
Anthropometric measurements		
BMI, kg/m^2, mean ± SD	22.7 ± 3.3	27.7 ± 6.2
Waist circumference, cm, mean ± SD (cm)	76.5 ± 9.4	88.3 ± 15.2
Hip circumference, cm, mean ± SD (cm)	96.3 ± 9.6	107.6 ± 12.7
Waist-to-hip-ratio, mean ± SD	0.80 ± 0.078	0.82 ± 0.076
Metabolic variables		
Systolic blood pressure, mmHg, mean ± SD	113.4 ± 13.08	120.1 ± 14.9
Diastolic blood pressure, mmHg, mean ± SD	74.1 ± 8.5	78.8 ± 8.8
Fasting glucose, mg/dL, mean ± SD	85.2 ± 10.2	93.6 ± 28.3
Total cholesterol, mg/dL, mean ± SD	197.9 ± 38.9	199.5 ± 43.5
High-density lipoprotein cholesterol, mg/dL, mean ± SD	77.1 ± 17.9	66.5 ± 17.4
Low-density lipoprotein cholesterol, mg/dL, mean ± SD	114.5 ± 33.6	121.9 ± 40.5
Triglycerides, mg/dL, mean ± SD	73 ± 26.8	102.3 ± 47
Metabolic syndrome, n (%)		
▪ No	145 (49.7%)	111 (38%)
▪ Yes	2 (0.7%)	34 (11.6%)
hs-CRP, mg/L, mean ± SD	1.48 ± 2.85	2.96 ± 3.69
Insulin, µU/mL, mean ± SD	7.04 ± 4.51	11.22 ± 8.85
HOMA-IR score ≥ 2.5, n (%)	14 (4.8%)	43 (14.7%)
Other variables		
MEDAS score (percentage of positively answered questions), mean ± SD	0.5 ± 0.16	0.47 ± 0.15
VO_{2peak}, ml/min/kg, mean ± SD	28.2 ± 6.3	23.0 ± 6.2
Ever smoked, n (%)		
▪ No	85 (29.1%)	62 (21.2%)
▪ Yes	67 (22.9%)	78 (26.7%)
Number of pack-years smoked, mean ± SD	3.7 ± 6.3	5.9 ± 9.4

after exercise) with the target of being exhausted with a respiratory exchange ratio (RER) > 1.05.

Statistical analysis

Women were categorized into high vs. low plasma bio-ADM based on the median levels in the entire cohort (< 13.8 and ≥ 13.8 pg/mL). Baseline statistics are presented as mean ± standard deviation or as median and range (continuous variables) or as proportions (binary and categorical variables). Logistic regression analysis was performed to estimate the odds ratios (OR) and their associated 95% confidence intervals (95% CI) of having high circulating bio-ADM levels by different cardiovascular risk factors. A multivariate analysis was carried out to control for potential confounders. These analyses were adjusted for age (years) and history of breast cancer (diseased or non-diseased).

Statistical significance was defined at the level of $p \leq 0.05$, and all analyses were carried out using SPSS version 25.0 (IBM Corp., Armonk, NY).

Results

There were 292 women with a *BRCA1* and/or *BRCA2* mutation included in the current study. ▶ **Table 1** summarizes selected participant characteristics by median bio-ADM levels. The median bio-ADM level was 13.8 pg/mL. The median age of the entire study cohort was 43 years (range: 18–72 years). Of all women, 57.5% had a previous diagnosis of breast cancer. The median time between breast cancer diagnosis and study entry was three years (range: 0–32 years). 19.6% of all participants had undergone PBSO. The

▶ **Table 2** Associations between bio-ADM levels (low vs. high) and selected patient characteristics among *BRCA* mutation carriers (univariate logistic regression).

Predictor	OR [95% CI]	p
Age	**1.026 [1.002; 1.050]**	**0.03***
BRCA mutation status	1.29 [0.82; 2.02]	0.27
Parity	1.2 [0.75; 1.9]	0.45
PBSO	1.32 [0.79; 2.21]	0.29
Age at PBSO	1.01 [0.95; 1.09]	0.69
Previous diagnosis of breast cancer	1.29 [0.81; 2.06]	0.28
Age at breast cancer diagnosis	1.02 [0.98; 1.06]	0.42
Time between breast cancer diagnosis and study entry	1.01 [0.96; 1.07]	0.71
Time between breast cancer diagnosis and study entry ≥ 4 years	**1.91 [1.00; 3.66]**	**0.05***
BMI	**1.28 [1,19; 1,37]**	**< 0.001***
Waist circumference	**1.09 [1.06; 1.11]**	**< 0.001***
Hip circumference	**1.10 [1.07; 1.14]**	**< 0.001***
Waist-to-hip-ratio	**49.22 [1.98; 1225.97]**	**0.02***
Systolic blood pressure	**1.04 [1.02; 1.06]**	**< 0.001***
Diastolic blood pressure	**1.07 [1.04; 1.1]**	**< 0.001***
Fasting glucose	**1.04 [1.02; 1.07]**	**< 0.001***
Total cholesterol	1.001 [0.995; 1.007]	0.74
High-density lipoprotein cholesterol	**0.97 [0.95; 0.98]**	**< 0.001***
Low-density lipoprotein cholesterol	1.005 [0.999; 1.012]	0.09
Triglycerides	**1.02 [1.02; 1.03]**	**< 0.001***
Metabolic syndrome	**22.21 [5.22; 94.42]**	**< 0.001***
hs-CRP	**1.19 [1.01; 1.41]**	**0.04**
Insulin	**1.13 [1.07; 1.19]**	**< 0.001***
HOMA-IR score ≥ 2.5	**4.01 [2.08; 7.72]**	**< 0.001***
MEDAS score (continuous)	0.28 [0.06; 1.4]	0.12
MEDAS score > 0.5	0.64 [0.41; 1.02]	0.06
VO_{2peak}	**0.88 [0.84; 0.92]**	**< 0.001***
Ever smoked	**1.72 [1.08; 2.74]**	**0.02***
Number of pack-years smoked	**1.04 [1.01; 1.07]**	**0.02***

* Results are statistically significant at a p value of ≤ 0.05 (in bold).

median age at PBSO was 45 years (range: 29–65 years). Tumour biology and breast cancer treatments were similar between the two groups.

Anthropometric variables between the two groups differed substantially: Women with low bio-ADM levels had a lower BMI and smaller waist and hip circumferences compared to women with high bio-ADM levels. Women among the high bio-ADM levels group had higher systolic blood pressure, higher diastolic blood pressure, higher fasting glucose levels, higher triglyceride levels, and lower HDL levels as compared to the low bio-ADM levels group. Thus, metabolic syndrome was more prevalent among women with high bio-ADM levels (11.6% vs. 0.7%). Peak oxygen uptake was substantially higher in the low bio-ADM levels group (28.2 ml/min/kg vs. 23.0 ml/min/kg). Among the high bio-ADM levels group, there were more women who have ever smoked (26.7% vs. 22.9%).

Univariate analysis

▶ **Table 2** summarizes the odds ratios (OR) and associated 95% confidence intervals (95% CI) of traditional cardiovascular risk factors associated with low vs. high bio-ADM levels among *BRCA1* and *BRCA2* mutation carriers. Increasing age was associated with a tendency to higher bio-ADM levels (OR = 1.03, p = 0.03). Bio-ADM levels were not associated with *BRCA* mutation status (p = 0.27), a previous history of breast cancer (p = 0.28) or PBSO (p = 0.29).

However, women who received their breast cancer diagnosis at least four years prior to study enrolment had higher odds of having increased bio-ADM levels (OR = 1.91, p = 0.05). Women fulfilling the criteria of metabolic syndrome had over 22-times higher odds of having increased bio-ADM levels compared to those who did not meet the criteria (OR = 22.2, p < 0.001). Moreover, higher bio-ADM levels were significantly associated with a bigger body size, as determined by BMI (OR = 1.28; p < 0.001), waist circumference (OR = 1.09; p < 0.001), hip circumference (OR = 1.1; p < 0.001), and waist-to-hip-ratio (OR = 49.22, p = 0.02). Moreover, high bio-ADM levels were associated with insulin resistance (OR = 4.01, p < 0.001) and higher hs-CRP levels (OR = 1.19, p = 0.04). Although not statistically significant, there was a trend which suggested that adaptation of the Mediterranean diet at baseline was associated with lower bio-ADM levels (OR = 0.64, p = 0.06). Cardiorespiratory fitness as indicated by peak oxygen uptake was associated with lower bio-ADM levels (OR = 0.88, p < 0.001). Bio-ADM levels were higher in women who have ever smoked (OR = 1.7; p = 0.02), and increased with the number of pack-years smoked (OR = 1.04; p = 0.02).

Multivariate analysis

Results were similar in the multivariate analysis adjusting for potential confounders including age and previous history of breast cancer (as described in ▶ **Table 3**).

Discussion

There is a need for early detection of subclinical cardiac dysfunction in breast cancer survivors. This need is not yet reflected in an effective screening program [5]. Several CVD risk scores have been investigated in the general population but were not found to be suitable for breast cancer survivors [13]. In current practice, echocardiography is the most widely used technique in the diagnosis, prevention and risk stratification of CVD before, during and after cancer treatment. Yet, there is no clear consensus on follow-up cardiac monitoring in breast cancer survivors. While conventional echocardiography can detect significant structural and functional changes, global left ventricular systolic function often

▶ **Table 3** Associations between bio-ADM levels (low vs. high) and selected patient characteristics among *BRCA* mutation carriers (multivariate logistic regression).

Predictor	OR [95% CI]	p
BRCA mutation status	1.26 [0.8; 2.0]	0.32
Parity	1.02 [0.61; 1.71]	0.95
PBSO	1.06 [0.78; 1,44]	0.69
Age at PBSO	0.996 [0.85; 1.17]	0.96
BMI	**4.33 [2.39; 7.85]**	**< 0.001***
Waist circumference	**1.08 [1.06; 1.11]**	**< 0.001***
Hip circumference	**1.1 [1.07; 1.14]**	**< 0.001***
Waist-to-hip-ratio	**31.1 [1.2; 805.8]**	**0.04***
Systolic blood pressure	**1.04 [1.02; 1.06]**	**0.001***
Diastolic blood pressure	**1.06 [1.03; 1.1]**	**< 0.001***
Fasting glucose	**1.04 [1.02; 1.07]**	**0.001***
Total cholesterol	0.999 [0.99; 1.01]	0.75
High-density lipoprotein cholesterol	**0.97 [0.95; 0.98]**	**< 0.001***
Low-density lipoprotein cholesterol	1.004 [0.997; 1.011]	0.26
Triglycerides	**1.02 [1.02; 1.03]**	**< 0.001***
Metabolic syndrome	**20.99 [4.91; 89.79]**	**< 0.001***
hs-CRP	**1.21 [1.02; 1.43]**	**0.03***
Insulin	**1.13 [1.07; 1.19]**	**< 0.001***
HOMA-IR score ≥ 2.5	**4.05 [2.09; 7.85]**	**< 0.001***
MEDAS score (continuous)	0.28 [0.06; 1.42]	0.124
MEDAS score > 0.5	0.63 [0.4; 1.01]	0.056
VO_{2peak}	**0.87 [0.83; 0.91]**	**< 0.001***
Ever smoked	**1.68 [1.05; 2.7]**	**0.03***
Number of pack-years smoked	**1.04 [1.00; 1.07]**	**0.04***

Adjusted for age (in years) and previous diagnosis of breast cancer (diseased vs. non-diseased).

* Results are statistically significant at a p value of ≤ 0.05 (in bold).

remains preserved until late in the course of CVD. Vasoactive peptides are directly related to the development and progression of CVD. Recent studies indicate that ADM might identify subclinical cardiac impairment prior to detectable changes in ejection fraction [43].

One way to make a screening program efficient is to apply it to a high-risk population. *BRCA1/2* mutation carriers are suggested to be at an increased risk for CVD, regardless of a previous cancer diagnosis [31, 33]. This is the first study to examine plasma bio-ADM levels among *BRCA* mutation carriers. In line with previous studies among the general population [44], high bio-ADM levels were associated with traditional cardiovascular risk factors, including age [45], BMI [45], insulin resistance [46, 47], metabolic syndrome [48], low cardiorespiratory fitness [49] and smoking [20, 50]. Central obesity (as measured by the waist-to-hip-ratio), rather than general obesity (as measured by BMI), was a strong predictor for high bio-ADM levels which corresponds to other investigations suggesting that adipose tissue is a major source of ADM [51 – 53]. Consistent with our findings, recent studies have shown that adipose tissue distribution outperforms BMI in identifying breast cancer survivors with a high risk for CVD [54]. As described previously in a cohort of cancer survivors [43, 50], we were able to confirm a significant association between the inflammatory marker hs-CRP and bio-ADM. Although not statistically significant in our baseline analysis conducted before intervention, there was a trend which suggested that adherence to the Mediterranean diet was associated with lower bio-ADM levels. After adjustment for age and history of breast cancer, the associations between bio-ADM levels and traditional cardiovascular risk factors remained stable.

Given its robust association with multiple CVD risk factors, our data suggest that bio-ADM might be useful in estimating the burden of CVD attributable to modifiable risk factors in *BRCA* mutation carriers.

ADM is an almost ubiquitously expressed peptide with vasodilatory and natriuretic properties. Previous studies have observed a link between high ADM levels and worse prognosis in patients with myocardial infarction and heart failure. With a prognostic value superior to that of brain natriuretic peptide [55], ADM plays a crucial role in the pathophysiology of major adverse cardiac events. More recently, studies among healthy individuals have shown that ADM levels become elevated years before the onset of CVD and cancer [16, 56]. Identification of the underlying mechanisms associated with this co-occurrence is of great public health importance.

Whilst ADM is a well-established biomarker for CVD, the role of ADM in breast cancer aetiology is less clear. ADM is expressed in sporadic breast cancer tissue [57, 58], and the degree of expression is associated with tumour growth [57, 59, 60], local tumour progression [58] and bone metastases [60, 61]. Preliminary evidence suggests that ADM influences the osteoclast differentiation mediated by Receptor Activator of NF-κB Ligand (RANKL) [61], an important signalling pathway in *BRCA1*-associated breast carcinogenesis [62, 63].

Contrary to expectations, history of breast cancer was not associated with elevated bio-ADM levels in our analysis. Nevertheless, we noted that women who were diagnosed with breast cancer at least four years before study entry had significantly higher bio-ADM levels, delineating them as a higher-risk cohort. Likewise, an older age was associated with a tendency to higher bio-ADM levels which might be attributable to longer oestrogen deprivation. However, due to the median age of the entire study cohort of 43 years, PBSO uptake was low in this population. Therefore, both PBSO and age at PBSO were not associated with higher bio-ADM levels. With respect to our study cohort, it is not entirely surprising that we found no association between circulating bio-ADM levels and history of breast cancer. In our cohort, the median time between breast cancer diagnosis and study entry was three years (range: 0–32 years), resulting in a selection bias for diseased women. Although this finding needs further confirmation, it is an interesting area of research with respect to the long latency periods between the initial diagnosis of breast cancer and the development of manifest CVD.

Strengths and limitations

Strengths associated with the current analysis include the comprehensive evaluation of cardiovascular risk factors using several objective measurements. After adjusting for age and prior history of breast cancer, the adjusted and unadjusted results did not differ significantly. Therefore, any additional confounding was likely small. Although our results provide an exciting direction for prevention research, this study had several limitations. The median age of our study cohort was 43 years. Thus, the prevalence of manifest CVD is expected to be low. The proportion of women who met the criteria of metabolic syndrome was 12.3% in our analysis. This compares to a prevalence of 18–21% among the general German population [64]. Considering the substantially lower prevalence of CVD risk factors among our study cohort, results obtained in this analysis likely underestimate the true associations between bio-ADM levels and outcomes attributable to modifiable risk factors. In order to estimate the association between ADM and traditional CVD risk factors, we used single measurements of bio-ADM at baseline only. Our study is limited by the fact that there is no reference cohort of *BRCA*-negative women. With regard to the lack of reference values for bio-ADM among the general population, we were not able to provide suitable bio-ADM thresholds for subclinical cardiac impairment. Pavo et al. have shown that patients with cancer and without prior cancer treatment had elevated levels of ADM even in the absence of overt CVD [43]. Although a continuous information would have been more informative and would have provided more decisive inference, we decided to dichotomize our outcome variable based on the median value of bio-ADM in order to increase robustness of our regression models. Given the prospective nature of the LIBRE trials, we will be able to elucidate the impact of a lifestyle intervention, namely physical activity and a healthy diet, on the change in bio-ADM levels over time. Finally, our cohort was not sufficiently powered to conduct analyses stratified by *BRCA* mutation type.

Conclusions

Identifying, monitoring and reducing CVD risk factors should be a priority for the long-term care of breast cancer survivors. Preliminary evidence suggest that *BRCA1/2* mutation carriers are more prone to CVD. In line with previous studies conducted in the general population, our results indicate that ADM is associated with several cardiovascular risk factors among *BRCA1/2* mutation carriers, irrespective of a previous breast cancer diagnosis. Further research is needed to define suitable bio-ADM thresholds for subclinical cardiac dysfunction. Moreover, the long-term clinical implications of reducing bio-ADM levels through lifestyle and/or medical interventions in women at high risk for breast cancer, complemented by mechanistic evidence, are yet to be determined.

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Trial registrations

NCT02087592; NCT02516540

Author Contributions Statement

JL conceptualized and designed the study, coordinated and conducted the acquisition and interpretation of data, carried out data analyses, and drafted the initial manuscript. MK conceived of the study, designed the study, coordinated the study and critically revised the manuscript. SG participated in the design of this study, was involved in the acquisition and interpretation of data, and gave final approval of the version to be published. JS and OH performed bio-ADM analyses and contributed to critical revision of the manuscript. MB, CE, SCB, ABE and MH were involved in the acquisition and interpretation of patient data and contributed to critical revision of the manuscript. All authors have read the manuscript and have given their final approval for publication of this study.

Ethics Statement

The study was approved by the institutional ethics review boards of both the host institutions (Technical University of Munich: Reference No. 5686/13, University Hospital Cologne: Reference No. 13-053 and University Hospital Schleswig-Holstein in Kiel: Reference No. B-235/13) and the participating study centers, and all study subjects provided written informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Data Availability Statement

Data is available upon reasonable request to the corresponding author.

Conflict of Interest

JS and OH are employed by Sphingotec GmbH, a company having patent rights in and commercializing the bio-ADM assay. MK and SG received grants from Sphingotec GmbH. JL, MB, CE, SCB, ABE and MH have nothing to disclose.

References

[1] Sturgeon KM, Deng L, Bluethmann SM et al. A population-based study of cardiovascular disease mortality risk in US cancer patients. Eur Heart J 2019; 40: 3889–3897

[2] Blaes AH, Konety SH. Cardiovascular Disease in Breast Cancer Survivors: An Important Topic in Breast Cancer Survivorship. J Natl Cancer Inst 2021; 113: 105–106

[3] Patnaik JL, Byers T, DiGuiseppi C et al. Cardiovascular disease competes with breast cancer as the leading cause of death for older females diagnosed with breast cancer: a retrospective cohort study. Breast Cancer Res 2011; 13: R64

[4] Kirkham AA, Beaudry RI, Paterson DI et al. Curing breast cancer and killing the heart: A novel model to explain elevated cardiovascular disease and mortality risk among women with early stage breast cancer. Prog Cardiovasc Dis 2019; 62: 116–126

[5] Mehta LS, Watson KE, Barac A et al. Cardiovascular Disease and Breast Cancer: Where These Entities Intersect: A Scientific Statement From the American Heart Association. Circulation 2018; 137: e30–e66

[6] Koelwyn GJ, Newman AAC, Afonso MS et al. Myocardial infarction accelerates breast cancer via innate immune reprogramming. Nat Med 2020; 26: 1452–1458

[7] Johnson CB, Davis MK, Law A et al. Shared Risk Factors for Cardiovascular Disease and Cancer: Implications for Preventive Health and Clinical Care in Oncology Patients. Can J Cardiol 2016; 32: 900–907

[8] Gernaat SAM, Ho PJ, Rijnberg N et al. Risk of death from cardiovascular disease following breast cancer: a systematic review. Breast Cancer Res Treat 2017; 164: 537–555

[9] Gernaat SAM, Boer JMA, van den Bongard DHJ et al. The risk of cardiovascular disease following breast cancer by Framingham risk score. Breast Cancer Res Treat 2018; 170: 119–127

[10] Park NJ, Chang Y, Bender C et al. Cardiovascular disease and mortality after breast cancer in postmenopausal women: Results from the Women's Health Initiative. PLoS One 2017; 12: e0184174

[11] Gulati M, Mulvagh SL. The connection between the breast and heart in a woman: Breast cancer and cardiovascular disease. Clin Cardiol 2018; 41: 253–257

[12] Gernaat SA, Isgum I, de Vos BD et al. Automatic Coronary Artery Calcium Scoring on Radiotherapy Planning CT Scans of Breast Cancer Patients: Reproducibility and Association with Traditional Cardiovascular Risk Factors. PLoS One 2016; 11: e0167925

[13] Abdel-Qadir H, Thavendiranathan P, Austin PC et al. Development and validation of a multivariable prediction model for major adverse cardiovascular events after early stage breast cancer: a population-based cohort study. Eur Heart J 2019; 40: 3913–3920

[14] Lyngbakken MN, Myhre PL, Rosjo H et al. Novel biomarkers of cardiovascular disease: Applications in clinical practice. Crit Rev Clin Lab Sci 2019; 56: 33–60

[15] Funke-Kaiser A, Havulinna AS, Zeller T et al. Predictive value of midregional pro-adrenomedullin compared to natriuretic peptides for incident cardiovascular disease and heart failure in the population-based FINRISK 1997 cohort. Ann Med 2014; 46: 155–162

[16] Melander O, Newton-Cheh C, Almgren P et al. Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. JAMA 2009; 302: 49–57

[17] Kawano S, Kawagoe Y, Kuwasako K et al. Gender-related alterations in plasma adrenomedullin level and its correlation with body weight gain. Endocr Connect 2015; 4: 43–49

[18] Vila G, Riedl M, Maier C et al. Plasma MR-proADM correlates to BMI and decreases in relation to leptin after gastric bypass surgery. Obesity (Silver Spring) 2009; 17: 1184–1188

[19] Ohlsson T, Nilsson PM, Persson M et al. Midregional proadrenomedullin predicts reduced blood pressure and glucose elevation over time despite enhanced progression of obesity markers. J Hypertens 2019; 37: 590–595

[20] Eggers KM, Venge P, Lindahl B et al. Associations of mid-regional pro-adrenomedullin levels to cardiovascular and metabolic abnormalities, and mortality in an elderly population from the community. Int J Cardiol 2013; 168: 3537–3542

[21] Kuchenbaecker KB, Hopper JL, Barnes DR et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA 2017; 317: 2402–2416

[22] Sajjad M, Fradley M, Sun W et al. An Exploratory Study to Determine Whether BRCA1 and BRCA2 Mutation Carriers Have Higher Risk of Cardiac Toxicity. Genes (Basel) 2017; 8: 59

[23] Hubner J, Katalinic A, Waldmann A et al. Long-term Incidence and Mortality Trends for Breast Cancer in Germany. Geburtshilfe Frauenheilkd 2020; 80: 611–618

[24] Metcalfe K, Gershman S, Lynch HT et al. Predictors of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. Br J Cancer 2011; 104: 1384–1392

[25] Metcalfe K, Lynch HT, Ghadirian P et al. Risk of ipsilateral breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat 2011; 127: 287–296

[26] Hodgson A, Turashvili G. Pathology of Hereditary Breast and Ovarian Cancer. Front Oncol 2020; 10: 531790

[27] Ditsch N, Stickeler E, Behrens A et al. Update Breast Cancer 2021 Part 2 – Advanced Stages, Long-Term Consequences and Biomarkers. Geburtshilfe Frauenheilkd 2021; 81: 539–548

[28] Schneeweiss A, Hartkopf AD, Muller V et al. Update Breast Cancer 2020 Part 1 – Early Breast Cancer: Consolidation of Knowledge About Known Therapies. Geburtshilfe Frauenheilkd 2020; 80: 277–287

[29] Gordon T, Kannel WB, Hjortland MC et al. Menopause and coronary heart disease. The Framingham Study. Ann Intern Med 1978; 89: 157–161

[30] Gordhandas S, Norquist BM, Pennington KP et al. Hormone replacement therapy after risk reducing salpingo-oophorectomy in patients with BRCA1 or BRCA2 mutations; a systematic review of risks and benefits. Gynecol Oncol 2019; 153: 192–200

[31] Arts-de Jong M, Maas AH, Massuger LF et al. BRCA1/2 mutation carriers are potentially at higher cardiovascular risk. Crit Rev Oncol Hematol 2014; 91: 159–171

[32] Gast KC, Viscuse PV, Nowsheen S et al. Cardiovascular Concerns in BRCA1 and BRCA2 Mutation Carriers. Curr Treat Options Cardiovasc Med 2018; 20: 18

[33] van Westerop LL, Arts-de Jong M, Hoogerbrugge N et al. Cardiovascular risk of BRCA1/2 mutation carriers: A review. Maturitas 2016; 91: 135–139

[34] Powell CB, Alabaster A, Armstrong MA et al. Risk of cardiovascular disease in women with BRCA1 and BRCA2 mutations. Gynecol Oncol 2018; 151: 489–493

[35] Shukla PC, Singh KK, Quan A et al. BRCA1 is an essential regulator of heart function and survival following myocardial infarction. Nat Commun 2011; 2: 593

[36] Singh KK, Shukla PC, Quan A et al. BRCA1 is a novel target to improve endothelial dysfunction and retard atherosclerosis. J Thorac Cardiovasc Surg 2013; 146: 949–960.e4

[37] Singh KK, Shukla PC, Quan A et al. BRCA2 protein deficiency exaggerates doxorubicin-induced cardiomyocyte apoptosis and cardiac failure. J Biol Chem 2012; 287: 6604–6614

[38] Kiechle M, Engel C, Berling A et al. Lifestyle intervention in BRCA1/2 mutation carriers: study protocol for a prospective, randomized, controlled clinical feasibility trial (LIBRE-1 study). Pilot Feasibility Stud 2016; 2: 74

[39] Kiechle M, Engel C, Berling A et al. Effects of lifestyle intervention in BRCA1/2 mutation carriers on nutrition, BMI, and physical fitness (LIBRE study): study protocol for a randomized controlled trial. Trials 2016; 17: 368

[40] Hebestreit K, Yahiaoui-Doktor M, Engel C et al. Validation of the German version of the Mediterranean Diet Adherence Screener (MEDAS) questionnaire. BMC Cancer 2017; 17: 341

[41] Marino R, Struck J, Maisel AS et al. Plasma adrenomedullin is associated with short-term mortality and vasopressor requirement in patients admitted with sepsis. Crit Care 2014; 18: R34

[42] Weber J, Sachse J, Bergmann S et al. Sandwich Immunoassay for Bioactive Plasma Adrenomedullin. J Appl Lab Med 2017; 2: 222–233

[43] Pavo N, Raderer M, Hulsmann M et al. Cardiovascular biomarkers in patients with cancer and their association with all-cause mortality. Heart 2015; 101: 1874–1880

[44] Neumann JT, Tzikas S, Funke-Kaiser A et al. Association of MR-pro-adrenomedullin with cardiovascular risk factors and subclinical cardiovascular disease. Atherosclerosis 2013; 228: 451–459

[45] Bhandari SS, Davies JE, Struck J et al. Influence of confounding factors on plasma mid-regional pro-adrenomedullin and mid-regional pro-A-type natriuretic peptide concentrations in healthy individuals. Biomarkers 2011; 16: 281–287

[46] Sahin I, Celik O, Celik N et al. Adrenomedullin: possible predictor of insulin resistance in women with polycystic ovary syndrome. J Endocrinol Invest 2012; 35: 553–556

[47] Lim SC, Morgenthaler NG, Subramaniam T et al. The relationship between adrenomedullin, metabolic factors, and vascular function in individuals with type 2 diabetes. Diabetes Care 2007; 30: 1513–1519

[48] Torres C, Lima-Martinez MM, Rosa FJ et al. [Epicardial adipose tissue and its association to plasma adrenomedullin levels in patients with metabolic syndrome]. Endocrinol Nutr 2011; 58: 401–408

[49] Kolditz M, Seyfarth HJ, Wilkens H et al. MR-proADM Predicts Exercise Capacity and Survival Superior to Other Biomarkers in PH. Lung 2015; 193: 901–910

[50] Krintus M, Kozinski M, Braga F et al. Plasma midregional proadrenomedullin (MR-proADM) concentrations and their biological determinants in a reference population. Clin Chem Lab Med 2018; 56: 1161–1168

[51] Nomura I, Kato J, Tokashiki M et al. Increased plasma levels of the mature and intermediate forms of adrenomedullin in obesity. Regul Pept 2009; 158: 127–131

[52] Del Ry S, Cabiati M, Bianchi V et al. Mid-regional-pro-adrenomedullin plasma levels are increased in obese adolescents. Eur J Nutr 2016; 55: 1255–1260

[53] Koyama T, Kuriyama N, Uehara R. Midregional Proadrenomedullin Can Reflect the Accumulation of Visceral Adipose Tissue-A Key to Explaining the Obesity Paradox. Int J Environ Res Public Health 2020; 17: 3968

[54] Cespedes Feliciano EM, Chen WY, Bradshaw PT et al. Adipose Tissue Distribution and Cardiovascular Disease Risk Among Breast Cancer Survivors. J Clin Oncol 2019; 37: 2528–2536

[55] Yuyun MF, Narayan HK, Ng LL. Prognostic significance of adrenomedullin in patients with heart failure and with myocardial infarction. Am J Cardiol 2015; 115: 986–991

[56] Daukantaite D, Tellhed U, Maddux RE et al. Five-week yin yoga-based interventions decreased plasma adrenomedullin and increased psychological health in stressed adults: A randomized controlled trial. PLoS One 2018; 13: e0200518

[57] Martinez A, Vos M, Guedez L et al. The effects of adrenomedullin overexpression in breast tumor cells. J Natl Cancer Inst 2002; 94: 1226–1237

[58] Oehler MK, Fischer DC, Orlowska-Volk M et al. Tissue and plasma expression of the angiogenic peptide adrenomedullin in breast cancer. Br J Cancer 2003; 89: 1927–1933

[59] Pare M, Darini CY, Yao X et al. Breast cancer mammospheres secrete Adrenomedullin to induce lipolysis and browning of adjacent adipocytes. BMC Cancer 2020; 20: 784

[60] Liu LL, Chen SL, Huang YH et al. Adrenomedullin inhibits tumor metastasis and is associated with good prognosis in triple-negative breast cancer patients. Am J Transl Res 2020; 12: 773–786

[61] Awolaran O, Brooks SA, Lavender V. Breast cancer osteomimicry and its role in bone specific metastasis; an integrative, systematic review of preclinical evidence. Breast 2016; 30: 156–171

[62] Nolan E, Vaillant F, Branstetter D et al. RANK ligand as a potential target for breast cancer prevention in BRCA1-mutation carriers. Nat Med 2016; 22: 933–939

[63] Sigl V, Owusu-Boaitey K, Joshi PA et al. RANKL/RANK control Brca1 mutation. Cell Res 2016; 26: 761–774

[64] Moebus S, Hanisch J, Bramlage P et al. Regional differences in the prevalence of the metabolic syndrome in primary care practices in Germany. Dtsch Arztebl Int 2008; 105: 207–213

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ORIGINAL ARTICLE

Outcomes of women at high familial risk for breast cancer: An 8-year single-center experience

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Abstract

Objectives: The value of a high-risk surveillance program for mutation carriers and women at high familial breast cancer risk has not been extensively studied. A Breast and Ovarian Cancer Risk Management Clinic (BOCRMC) was established at the Royal Melbourne Hospital in 2010 to provide multimodality screening and risk management strategies for this group of women. The aims of this study were to evaluate the program and describe breast cancer diagnoses for *BRCA1*, *BRCA2*, and other germline mutation carriers as well as high-risk noncarriers attending the BOCRMC.

Methods: Clinical data from mutation carriers and noncarriers with a ≥25% lifetime risk of developing breast cancer who attended between 2010 and 2018 were extracted from clinic records and compared. The pattern and mode of detection of cancer were determined.

Results: A total of 206 mutation carriers and 305 noncarriers attended the BOCRMC and underwent screening on at least one occasion. Median age was 37 years. After a median follow-up of 34 months, 15 (seven invasive) breast cancers were identified in mutation carriers, with seven (six invasive) breast cancers identified in noncarriers. Of these, 20 (90.9%) were detected by annual screening, whereas two (9.1%) were detected as interval cancers (both in *BRCA1* mutation carriers). Median size of the invasive breast cancers was 11 mm (range: 1.5-30 mm). The majority (76.9%) were axillary node negative. In women aged 25-49 years, the annualized cancer incidence was 1.6% in *BRCA1*, 1.4% in *BRCA2* mutation carriers, and 0.5% in noncarriers. This compares to 0.06% annualized cancer incidence in the general Australian population.

Conclusions: Screening was effective at detecting early-stage cancers. The incidence of events in young noncarriers was substantially higher than in the general population. This potentially justifies ongoing management through a specialty clinic, although further research to better personalize risk assessment in noncarriers is required.

KEYWORDS

BRCA1 gene, BRCA2 gene, breast cancer screening, early detection of cancer, hereditary breast and ovarian cancer syndrome

1 | INTRODUCTION

Breast cancer screening and preventive measures should ideally be tailored to an individual's risk profile to ensure that the cost/benefit ratio of the selected option is favourable.[1]

A family history of breast cancer is the most widely recognized risk factor: approximately 10-15% of breast cancers are assumed to be familial, with 25% of these being associated with a mutation in a high-penetrance hereditary breast cancer gene.[2,3] Hence, the majority of women with a substantial family history for breast cancer will

test negative for a genetic mutation. Among this group, the clinical value of genetic modifiers identified through genome-wide association studies (GWAS)[4] has yet to impact on patient risk assessment and management.[5]

An international collaborative study that included Australian women found that the cumulative breast cancer risk by age 80 years was 72% for *BRCA1* and 69% for *BRCA2* mutation carriers. Breast cancer risk for *BRCA* mutation carriers increased rapidly with age in early adulthood, then plateaued to a relatively constant rate. The peak hazard ratio for breast cancer risk occurred in the 30s for *BRCA1* and in the 40s for *BRCA2* mutation carriers.[6] Although guidelines vary across countries, *BRCA1/2* mutation carriers (and women with a known hereditary cancer syndrome) are generally encouraged to participate in dedicated high-risk clinics that offer regular breast cancer screening and risk-reducing options. For the larger group of women at high familial risk of breast cancer where no heritable germline mutation has been identified, the optimal approach in managing their cancer risks is unknown.[7] Cancer Australia provides a familial risk assessment online tool, FRA-BOC,[8] to determine an unaffected woman's risk of developing breast cancer based on her family history that categorizes the risk level as 1 (representing general population risk), 2 (moderately increased risk), or 3 (potentially high risk). Category 3 (estimated to approximate to >25% lifetime risk) includes women

1. having two first degree or second degree relatives on one side of the family diagnosed with breast or ovarian cancer plus one or more of the following features on the same side of the family: additional relative(s) with breast or ovarian cancer, breast cancer diagnosed before the age of 40, bilateral breast cancer, breast and ovarian cancer in the same women, Jewish ancestry, and/or breast cancer in a male relative,
2. having a family member with a known mutation predisposing to breast cancer, and
3. who are at potentially high risk of ovarian cancer.

A detailed description of the FRA-BOC classification is provided in the addendum.

Since 1 February 2009, breast magnetic resonance imaging (MRI) screening has been subsidized by Medicare Australia for annual surveillance in women under age 50 who are at high risk for breast cancer.[9] The Australian Government funds annual breast MRI screening for "the diagnosis of breast cancer in asymptomatic women with a high risk of developing breast cancer when used as part of an organized surveillance program." Subsidized access to this test is provided to women under the age of 50 who tested positive for a genetic mutation that predisposes to breast cancer or to mutation-negative women who fit the FRA-BOC category 3.[10]

The Breast and Ovarian Cancer Risk Management Clinic (BOCRMC) was established in August 2010 at the Royal Melbourne Hospital to centralize the assessment and management of high-risk women. This is conducted in conjunction with the Gynaecological Oncology Service at the Royal Women's Hospital, Melbourne, for ovarian cancer preventive surgery. Initially, referral criteria for the BOCRMC were in accordance with the category 3 FRA-BOC classification as proposed by Cancer Australia and/or with eligibility for Medicare coverage of surveillance MRI. From late 2015, referral criteria were defined as a lifetime risk of developing breast cancer of at least 25% based on the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA)[11] and/or MRI eligibility as per category 3 of the FRA-BOC. The clinic provides regular breast screening and clinical breast examinations (CBEs), and informs patients about risk-reducing strategies including bilateral prophylactic mastectomy (BPM),[12] bilateral risk-reducing salpingo-oophorectomy,[1] endocrine preventive strategies,[1,13] and lifestyle recommendations.[14]

The aim of this study was to evaluate the current approach to patient selection in the BOCRMC, and to investigate whether the reference criteria should be restricted to mutation carriers only, or should remain more broad. Therefore, we assessed the clinical outcomes of high-risk women attending the BOCRMC with regard to breast malignancies detected through the screening services provided and their uptake rate of preventive options, and described these outcomes in both mutation carriers and noncarriers.

2 | METHODS

A retrospective review was performed using prospectively collected data from women attending the BOCRMC at the Royal Melbourne Hospital between 3 August 2010 and 31 July 2018. These patients were referred from familial cancer centers, public and private breast clinics, and general practitioners.

Initially, high risk was broadly defined as eligibility for Medicare coverage of surveillance MRI and/or category 3 of the FRA-BOC classification. Eligible subjects included both carriers of a pathogenic *BRCA1*, *BRCA2*, *CDH1*, *MSH6*, *TP53*, *PALB2*, *PTEN*, or *STK11* mutation and noncarriers.

The BOADICEA tool[11] was introduced in late 2015 to further estimate the lifetime risks of breast cancer in women attending clinic with no known mutation in a cancer predisposing gene.

At the BOCRMC, breast surgeons discuss the available breast cancer risk management options with the patient. A personalized risk management plan is devised based on the mutation status, age, youngest age of cancer diagnosis in the family, and any medical condition that might affect the screening modality. The screening methods utilized in the clinic include yearly CBE and breast ultrasound from age 25 years, yearly breast MRI for women aged between 25 and less than 50 years, and mammogram from age 30 years. Self-funded MRI screening is offered to women aged 50 years or older. In addition, other options are discussed with the patient, including prophylactic surgeries, for example, bilateral mastectomy and risk-reducing bilateral salpingo-oophorectomy (after child bearing is completed), lifestyle recommendations (weight management, physical activity, diet, smoking, and alcohol intake),[15] and, if eligible, risk-reducing medication[16] and clinical trials (eg, the BRCA-D study).[17]

For this study, de-identified data were extracted from the local clinical database FamBIS (Cancer Council Victoria, version 3.8.1), and entered into a separate study database with institutional ethics approval.

Demographics, personal and family cancer history, genetic test results, screening history, and risk reduction data were collected. Within the follow-up period of up to 8 years, data included any new cancers detected within the BOCRMC, including detection modality, tumor size, pathology and therapies, updated information on the uptake of risk-reducing services, and if applicable, date and reason for discharge from the clinic.

The study was approved by the Human Research Ethics Committee of the Royal Melbourne Hospital.

All data were analyzed in a descriptive fashion using R version 3.5.2 (https://www.R-project.org/).

3 | RESULTS

Between August 2010 and July 2018, a total of 511 women attended the BOCRMC at the Royal Melbourne Hospital for breast cancer surveillance due to the presence of a genetic mutation or strong personal family history.

The patient characteristics are shown in Table 1. The median age of the study population was 37 years (range 18-70 years) at the time of their first consultation. The median follow-up time within the clinic was 34 months (range 1-96 months).

Three hundred and five women (59.7%) had no known germline mutation, but had a strong family history of breast and/or ovarian cancer. Of these, 296 women tested negative for a genetic mutation. Based on their family history, these women were offered genetic testing for mutations in the *BRCA1* or *BRCA2* genes from 2010 to 2012, and panel

TABLE 1 Characteristics of 511 women at the time of their first consultation in the BOCRMC

Median age (range)		
37 years (18-70 years)		
Median follow-up time (range)		
34 months (1-96 months)		
Mutation status	**Number of patients**	**Additional information**
BRCA1	90	One subject had a *BRCA2* variance of unknown significance (VUS) at the same time.
BRCA2	99	One subject had a concomitant pathogenic *MSH2* mutation.
CDH1	3	
MSH6	1	
STK11	6	
PALB2	2	
PTEN	1	
TP53	4	
None detected	305	Lifetime risk of developing breast cancer in N = 201, based on the risk model BOADICEA, median: 20%, range: 10.7-40%
Women tested negative for a mutation	296	
Women who did not undergo genetic testing	9	Four refused to undergo predictive genetic testing, two index patients did not receive genetic testing (one refused, one lived overseas), three family members were tested positive for a likely benign variant of uncertain significance (VUS) in the *BRCA1* or *BRCA2* genes—no predictive genetic testing was offered for the VUS
Pathology of previously diagnosed cancers in 36 patients	**Number of previous cancers**	**Number of previous cancers by mutation status**
Breast cancer	7 × invasive ductal carcinomas (IDC)	4 × *BRCA1* (one had a *BRCA2* VUS at the same time), 1 × *BRCA2*, 1 × *TP53* (bilateral breast cancer)
	1 × invasive lobular carcinoma (ILC)	1 × *BRCA2*
	2 × lobular carcinomas in situ (LCIS)	2 × noncarriers
	2 × ductal carcinomas in situ (DCIS)	2 × noncarriers
Ovarian cancer	21	14 × *BRCA1*
		7 × *BRCA2*
Endometrial cancer	1	1 × *PALB2*
Multiple myeloma	1	1 × *BRCA2*
Mucoepidermoid cancer of the lung	1	1 × *BRCA2*
Cervical cancer	1	1 × noncarrier

genetic testing for mutations in *BRCA1*, *BRCA2*, *TP53*, and *PTEN* from 2012 to 2016. The panel was extended by the ATM genes in 2017. Nine patients were not tested for a genetic mutation due to the following reasons: four refused to undergo predictive genetic testing after a genetic mutation was detected in a family member, one index patient refused genetic testing—consequently, the unaffected patient was not tested, one index patient lived in Nepal and did not receive subsidized genetic testing—consequently, the unaffected patient was not tested, the family members of three patients were tested positive for a likely benign variant of uncertain significance (VUS) in the *BRCA1* and *BRCA2* genes—no predictive genetic testing for the VUS was offered. As the subgroup of women who did not undergo genetic testing (n = 9/305) was negligibly small, we did not treat them separately from the women who tested negative for a deleterious mutation, but included them in the "noncarriers" group. BOADICEA scores for lifetime risk estimates of breast cancer were calculated for 201 of the 305 (65.9%) women. The median BOADICEA lifetime risk of developing breast cancer in this subgroup was 20% (range 10.7-40%). BOADICEA scores were not calculated for the remaining 104 (34.1%) women, mostly (82, 78.8%) because they had been discharged from the clinic before 2016, that is, before the algorithm was introduced. For this study, we decided to not calculate the BOADICEA scores retrospectively in already discharged patients because it would not have changed their subsequent risk management.

The remaining 206 women (40.3%) were carriers of a pathogenic mutation in breast cancer and/or ovarian cancer predisposition gene. Germline mutations were as follows: *BRCA1* (n = 90), *BRCA2* (n = 99), *STK11/LKB1* (Peutz Jeghers Syndrome, n = 6), *CDH1* (n = 3), *TP53* (Li-Fraumeni Syndrome, n = 4), *MSH6* (n = 1), *PALB2* (n = 2), and *PTEN* (Cowden Syndrome, n = 1). The *STK11*, *CDH1*, and *MSH6* carriers were also undergoing separate gastrointestinal surveillance.

Thirty-six study subjects already had a personal history of cancer when entering the BOCRMC, including 12 breast cancers and 21 ovarian cancers (Table 1).

3.1 | Discharges from the clinic

To date, 210 women have been discharged from the clinic (Table 2). Of the women discharged, 55 (26.2%) were discharged due to the completion of risk-reducing surgeries and 53 (25.2%) due to not being at sufficiently high risk after re-evaluation of their family pedigree in subsequent screening rounds (eg, corrections to the number of cancer cases in the family or the age of disease onset in a family member) or after a negative predictive genetic test result became available. These women were then referred to BreastScreen Australia's recommended screening interval of 2 years. Twenty-five women (11.9%) were discharged from the BOCRMC due to the transfer to oncologic care because of a cancer diagnosis. During the study period, four *BRCA1* mutation carriers died from cancer. Two had a recurrent ovarian cancer after 2 and 5 years, respectively, and two developed brain metastases 1.5 and 5 years, respectively, after their breast cancer diagnosis and deceased shortly after. One patient self-discharged from the clinic as she decided to opt out of regular surveillance and preventive surgery.

TABLE 2 Reasons for discharge from the clinic (N = 210)

Reason for discharge	Number of patients
Not at high risk	53 (*BRCA* predictive testing resulted in a negative test result in 10 subjects)
Prophylactic surgeries	55
Managed elsewhere	21
Transfer to oncologic care due to a recent cancer diagnosis	16 × invasive carcinoma
	8 × DCIS
	1 × LCIS
Deceased (due to cancer)	4
Missed appointments	28
Moved interstate/overseas	19
Self-discharged due to high cancer anxiety	1
To be re-referred	4

The remaining 72 (34.3%) were discharged from the clinic for other reasons (ie, personal decision not to continue with management in the clinic, missed appointments, managed elsewhere, and patient moved interstate or overseas).

3.2 | Cancer diagnoses

Twenty-two breast malignancies and two fallopian tube cancers were detected within the BOCRMC. Outside of the BOCRMC, another five cancers were detected in three patients: one in situ melanoma in a noncarrier, three unrelated primary cancers (metastatic uterine leiomyosarcoma, rectal adenocarcinoma, and primary lung adenocarcinoma) detected in a *TP53* mutation carrier, and one metastatic gastric cancer (regular gastroscopies were being conducted with gastroenterologist oversight) detected in a *CDH1* mutation carrier (data not shown).

The pathologic features of the breast cancer cases detected during the study period are detailed in Table 3.

The breast cancers included nine ductal carcinomas in situ (DCIS), 12 invasive ductal cancers (IDC), and one invasive lobular cancer. Of the 22 breast malignancies detected in the series, 15 occurred in mutation carriers and seven in noncarriers. The median BOADICEA lifetime risk for developing breast cancer in the seven noncarriers was only 14.7% (range: 12.4–26.2%). One *BRCA2* mutation carrier had two metachronous tumors (DCIS diagnosed in 2013 and 2016).

In women aged 25-49 years, the annualized breast cancer incidence rate was 1.6% in *BRCA1* and 1.4% in *BRCA2* mutation carriers, compared to 0.5% in noncarriers. Limiting the group of noncarriers to women who were tested negative for a genetic mutation, the annualized breast cancer incidence rate was 0.6% in mutation-negative women between ages 25 and 49 years.

Twenty of the 22 breast cancers were detected during annual screening. All screen-detected cancers were incident cases. The median number of screening rounds at which the breast cancers were

TABLE 3 Characteristics (at time of diagnosis) of the 22 breast malignancies detected in the BOCRMC between August 2010 and July 2018

Cancer type	*BRCA1*	*BRCA2*	*STK11*	
DCIS (N = 9 in 8 subjects) • Six high-grade DCIS • Three intermediate-grade DCIS	2 (one was previously diagnosed with stage IV ovarian cancer)	3 (one had two metachronous diagnoses; one had a *MSH2* mutation at the same time)	2	
IDC (N = 12)	3	3	0	
ILC (N = 1)	0	1	0	
Grade of invasive cancers (N = 13)				
	BRCA1	*BRCA2*		
Grade 1	0	0		
Grade 2	2	2		
Grade 3	2	2		
Axillary lymph node involvement				
	BRCA1	*BRCA2*		
Positive	0	1 Positive nodes/all extirpated nodes: 4/12		
Negative	4	3		
Receptor status of invasive cancers (N = 13)				
	BRCA1	*BRCA2*		
Triple-negative, basal-like	3	0		
ER positive, PR positive, HER2 negative	1	4		
ER positive, PR negative, HER2 negative	0	0		
ER positive, PR positive, HER2 amplified	0	0		
Surgical treatment				
	BRCA1	*BRCA2*	*STK11*	
Lumpectomy only	0	2 (one had two distinct diagnoses of DCIS; one had a *MSH2* mutation at the same time)	0	
Mastectomy	5	5	2	
Breast cancer detection methods				
	MRI	Mammogram	Ultrasound	Self-detected
Total numbers of tumors detected by each method	12 (12)	12 (18)	7 (8)	1 (1)
Women aged under 50 years (n = 15 women)	12 (12)	8 (11)		1
Women aged at least 50 years (n = 6 women, 7 cancers)	0	4 (7)	5 (6)	0
Number of screening rounds at which the breast cancer was detected				
	BRCA1	*BRCA2*	*STK11*	Noncarrier
Median (range)	3 (2-6)	4 (3-7)	6.5 (6-7)	4 (2-8)

detected was 4 in mutation carriers (range: 2-7 rounds) and 4 in non-carriers (range: 2-8 rounds). Thirteen breast cancers were detected in women before age 50 years, and nine breast cancers were detected in eight women aged at least 50 years. In the 13 women diagnosed before age 50 through annual screening, MRI detected 100% (12/12) of the cases and mammogram 72.7% (8/11). Seven out of nine DCIS were diagnosed in women aged under 50. Of these, all (7/7) DCIS were detected by MRI. Mammography failed to identify DCIS in one of these women. In women aged 50 or older, mammogram identified 57.1% (4/7) of breast cancer cases. The remaining 42.9% (3/7) were detected by ultrasound. As no (self-funded) breast MRI scans were undertaken in the patients ≥50 years of age who developed breast cancer, its sensitivity could not be documented for this group. MRI recall rates have been previously reported at our institution, where

we found that recall rates decreased substantially with increasing experience.[18]

For the two interval breast cancers, one was detected as an incidental finding in a prophylactic mastectomy specimen in a *BRCA1* mutation carrier and the other *BRCA1* mutation carrier presented as a self-detected axillary lump during pregnancy 10 months after her last screening MRI.

The two fallopian tube cancers were incidental findings made at the time of prophylactic surgery and elective surgery undertaken to treat uterine prolapse. Both cancers were detected at an early stage (1A and 1B).

The median age at breast cancer diagnosis was 45.5 years (range 28-60 years). The median age at diagnosis for the 14 mutation carriers was 45 years (range 28-60 years) and for the seven noncarriers was 48 years (range 35-60 years).

In five cases (22.7% overall), breast conserving surgery was chosen, whereas the majority (17, 77.3%) of cases proceeded with mastectomy.

The median size of the invasive breast cancers was 11 mm (range 1.5-30 mm). In total, 69.2% of the invasive breast cancers were 15 mm or smaller. The majority (61.5%, 8/13) of these tumors were low to intermediate grade. At time of diagnosis, 3/13 (23.1%) patients with invasive breast cancer had axillary lymph node metastases (one mutation carrier and two noncarriers).

Phenotypic evaluation of the 13 invasive tumors by IHC and HER2 CISH revealed that three were triple-negative, basal-like breast cancers (all *BRCA1* mutation carriers), eight were hormone receptor-positive and HER2 nonamplified (one *BRCA1*, two *BRCA2* mutation carriers, and five noncarriers), and two were hormone receptor positive and HER2-amplified (noncarriers only).

Although all cases presented as early breast cancer, two patients experienced distant recurrence on long-term follow-up. Patient 1 was first diagnosed with a *BRCA1*-associated, 8 mm Grade 3 triple-negative, node-negative breast cancer. Despite undergoing bilateral mastectomy and adjuvant chemotherapy, she presented with regional axillary node metastasis (10/28 lymph nodes) 2.5 years later, treated by axillary dissection and chemotherapy. She was subsequently diagnosed with brain metastases and died 2 years later. The second patient was first diagnosed with a *BRCA1*-associated 16 mm Grade 3 basal-like triple negative breast cancer during pregnancy, with no involved axillary nodes. She was treated with wide excision, adjuvant radiotherapy, and chemotherapy. She developed a second ipsilateral Grade 3 basal-like triple negative tumor 9 months later, presumed to be a local recurrence. She was then treated with bilateral total mastectomies and chemotherapy. She developed bone and brain metastases and died shortly after.

3.3 | Risk-reducing surgery

A total of 12.6% of all unaffected women elected to undergo BPM and 13.1% risk-reducing bilateral salpingo-oophorectomies (BSO; Table 4). The majority of these were *BRCA1* or *BRCA2* mutation carriers. A total of 37.5% (24/64) of the *BRCA1* mutation carriers underwent BPM at a median age of 39 and 19.2% (15/78) of the *BRCA2* mutation carriers underwent BPM at a median age of 39. Of the *BRCA1* mutation carriers who had BPM, 33.3% (8/24) were 35 years or younger at the time of the procedure. Of all *BRCA2* mutation carriers who underwent BPM, 73.3% (11/15) were ≤45 years old at the time of BPM. Overall 5.7% (16/283) of noncarriers underwent BPM at a median age of 44.5 years.

Out of 65 unaffected patients who were *BRCA1*-positive, 30.8% (20/65) had a BSO at a median age of 46 years. Until age 45 years, 18.5% (10/54) of the unaffected *BRCA1* mutation carriers underwent a BSO. Of all unaffected *BRCA2* mutation carriers, 26.6% (21/79) had a BSO at a median age of 45 years. Until age 45 years, 18.8% (12/64) of the *BRCA2* mutation carriers had a BSO conducted. A total of 5.3% (15/283) of the noncarriers underwent BSO at a median age of 43 years. All noncarriers who proceeded with a BSO had a family history of ovarian cancer.

3.4 | Endocrine medical prevention

As previously reported, all patients attending the BOCRMC between February 2014 and May 2015 received evidence-based information on endocrine preventive therapy.[16] There was a 6.8% collective uptake of tamoxifen (Table 4). Among the *BRCA1* mutation carriers, 1.5% (1/65) opted for chemoprevention. Among the *BRCA2* mutation carriers, 8.9% (7/79) decided to initiate chemoprevention. These women

TABLE 4 Prophylactic surgery and preventive medicine uptake cancer-free women

	Proportion of patients by mutation status			
Prophylactic measurement	***BRCA1***	***BRCA2***	***PTEN***	**Noncarrier**
Bilateral prophylactic mastectomy (BPM)	36.9% (24/65)	19% (15/79)	100% (1/1)	5.7% (16/283)
Median age (range) in years	39 (27-59)	39 (20-53)	53	44.5 (26-60)
	BRCA1	*BRCA2*	*STK11*	Noncarrier
Bilateral risk-reducing salpingo-oophorectomy (BSO)	30.8% (20/65)	26.6% (21/79)	50% (2/4)	5.3% (15/283)
Median age (range) in years	45.5 (37-55)	45 (34-68)	38 (33-43)	43 (39-59)
BSO conducted at age ≤45 years	18.5% (10/54)	18.8% (12/64)	66.7% (2/3)	5.3% (11/209)
	BRCA1	*BRCA2*	*CDH1*	Noncarrier
Tamoxifen (1-60 months)	1.5% (1/65)	8.9% (7/79)	50% (1/2)	7.4% (21/283)
Median age (range) in years	42	42 (33-58)	46	43.8 (31-67)

went on tamoxifen at a median age of 42 years. Of 283 noncarriers, 7.4% took tamoxifen for chemoprevention at a median age of 44 years. Most patients had a short duration of tamoxifen uptake, i.e. less than a year.

4 | DISCUSSION

Although risk management protocols for *BRCA* mutation carriers are well defined,[18] the optimal approach to managing the cancer risks of women who have a strong family history, but no known familial mutation for breast cancer, remains uncertain. The impact of intensified screening, prophylactic surgeries, and chemoprevention has not been extensively studied,[7] meaning that best practice for the care of this population is unclear.

The main goal of a breast cancer screening program is to identify breast cancers at the earliest stage possible due to its association with superior prognosis. DCIS is a nonobligate precursor, with an extremely low breast cancer specific mortality.[19] The natural history of untreated DCIS is not fully understood, but almost all high and intermediate grade lesions and a majority of low grade lesions will eventually progress to invasive cancer,[20] so prevention of breast cancer by diagnosis and treatment of DCIS is an important objective of a screening program in younger women. Death from breast cancer after a diagnosis of DCIS is substantial for women diagnosed before age 35 years.[19]

Therefore, we report on the breast cancer incidence rate including both invasive cancers and DCIS.

Among the general population, the annualized breast cancer incidence rate in women aged 25-49 years is approximately 0.06%.[21,22] This compares to 1.6% in *BRCA1* and 1.4% in *BRCA2* mutation carriers and 0.5% in noncarriers at the BOCRMC.

4.1 | Breast cancer risk assessment

Risk definitions and management practices are highly variable across countries. In Australia, "high risk" is defined as a lifetime risk of 25% or greater, which is captured by the FRA-BOC category 3,[8] whereas, in contrast, some countries apply other thresholds (eg, American Cancer Society: lifetime risk ≥20%; United Kingdom: lifetime risk ≥30% according to the National Institute of Health Care Excellence).[1]

Furthermore, internationally, neither guideline clearly specifies which empirical model to use to calculate a woman's risk of developing breast cancer; rather, a number of applicable risk models are recommended to estimate a woman's lifetime and age-specific risk of developing breast cancer,[23] applying indicators in various detail (family history ± genetic factors, and sometimes nongenetic factors) and weighing them differently. Internationally, there is no clear consensus on which risk estimation model is the most accurate and reliable, and comparative evaluations of different models showed minimal agreement in the assigned risk estimates.[24,25] Ozanne et al used the BRCAPRO, Claus, and Tyrer-Cuzick calculators to determine a woman's eligibility for breast MRI screening based on a lifetime risk of ≥20%.[24] The percentage of women identified by the Tyrer-Cuzick model was 5.6%, by BRCAPRO was 0.4% and by the Claus model was 0.9% using data from 10 000 women. Only 0.2% of the study population was found to be eligible by all three methods. Quante et al comparatively evaluated the performance of the risk models IBIS and BOADICEA to determine a woman's eligibility for MRI screening (lifetime risk of ≥20%).[25] Based on data from 1764 women, IBIS identified 59.3% of women to be eligible, compared to only 20.1% identified by BOADICEA.

For the selection of patients in Australia, Cancer Australia recommends to use category 3 of the FRA-BOC. Although the FRA-BOC is easy to apply in the clinical setting, it is a large category and, therefore, may result in underestimating risk in small sized families with few at-risk female relatives or paternally inherited risk, and overestimating risk in large families with multiple at-risk female relatives. Furthermore, the online tool does not incorporate tumor pathology. BOADICEA has been validated in an Australian population of European ancestry earlier,[26] and was introduced to the BOCRMC in late 2015. The BOADICEA risk score was calculated for 201 mutation-negative women. Using BOADICEA, only 22.4% (45/201) would have been classified as high risk using the FRA-BOC threshold of ≥25%. Based on a lifetime risk of ≥20% (as proposed by the American Cancer Society), the proportion of eligible women would equal 50.7% (102/201) using BOADICEA. Thus, a substantial number of mutation-negative women would not have met the inclusion criteria basing breast cancer risk status on BOADICEA only. Although BOADICEA was introduced to tailor breast cancer risk better, six out of seven mutation-negative women who had screen-detected breast cancers met the FRA-BOC criteria, but not the BOADICEA lifetime risk of at least 25%. Of these six cases, four women were diagnosed with breast cancer (3 × IDC, 1 × DCIS) before age 50 years (median age: 38 years, range: 35-48 years). If BOADICEA is likely to underestimate cancer risk in these individuals, a combination of validated risk models may be useful in reducing misclassification bias as proposed by Park et al.[27] In order to help with choosing the right breast cancer risk estimation model, the web-based decision support tool iPrevent uses initial questions to determine whether IBIS or BOADICEA is best to use, thereby providing a more accurate risk assessment at the individual level.[28] Mavaddat et al have recently published a polygenic risk score including 313 Single Nucleotide Polymorphisms (SNPs), based on the largest available GWAS dataset (derived from white European populations) and optimized for prediction of ER-positive and ER-negative disease. The polygenic risk score has the potential to improve risk stratification for screening and prevention strategies, and may be incorporated into risk prediction models, for example, BOADICEA. Clinical translational studies are necessary to evaluate the predictive value of the new PRS_{313} combined with family history and lifestyle risk factors in the context of current screening protocols.[29]

4.2 | Multimodality screening for breast cancer

The addition of MRI to mammography is beneficial in the early detection of breast cancer in high-risk individuals.[30–34] MRI has consistently been shown to outperform mammography and ultrasound with

regard to sensitivity, at the cost of reduced specificity.[30] In this series, MRI was superior to mammography (sensitivity of 100% vs 72.7%) in the detection of breast cancers in women aged under 50. In line with the EVA trial,[35] MRI was superior to mammography for diagnosing DCIS (100% vs 83.3%) among women less than 50 years of age. Kuhl et al suggest that if the findings of the EVA trial are replicated in empirical studies, it may be conceivable to discontinue mammographic screening in young women who regularly attend quality-assured screening with breast MRI.[35] There is some evidence to suggest that this might hold true in *BRCA1* mutation carriers under age 40.[30]

4.3 | Surgical prevention and chemoprevention

Among *BRCA1/2* mutation carriers, BPM reduces the risk of breast cancer by at least 90%,[12] and Ingham et al indicated that any risk-reducing surgery conducted in *BRCA* mutation carriers reduced mortality.[36] A simulation study suggested that although there was a substantial mortality benefit of BPM for *BRCA1* mutation carriers at age 25 years, the expected benefit declined rapidly with increasing age at BPM.[37] Phillips et al assessed the rate of BPM and BSO in Australian *BRCA* mutation carriers.[38] At 3-year follow-up from genetic testing, 5.2% (7/134) had undergone BPM at a mean age of 40 and 16.3% (20/123) had undergone BSO at a mean age of 47 years. Metcalfe et al reported on large differences in the uptake of BPM according to country of residence, with uptake rates varying from 2.7% in Poland to 36.3% in the United States.[39] The BPM uptake rate of 27.1% in *BRCA* mutation carriers in our study cohort is comparable to Western European countries (eg, France: 25%), but is higher than the rate reported by Phillips et al, including Australian women who indicated their uptake of BPM and BSO between 2001 and 2005. Several researchers, for example, James et al,[40] Evans et al,[41] and Liede et al,[42] have shown that after Angelina Jolie disclosed that she is a *BRCA1* mutation carrier and opted to have a BPM, there was a significant, long-lasting, and global increase in genetic testing and mastectomy rates since 2013. The different analysis periods may have had an impact on the BPM and BSO rates. Considering the reduction in clinical benefit of BPM with increasing age, the fact that 66.7% of the *BRCA1* mutation carriers who underwent BPM at the BOCRMC were older than 35 years at the time of the procedure is of concern.

Evans et al reported on the uptake of risk-reducing surgery among 3515 women with no known *BRCA1/2* mutation from England.[43] After a median follow-up of 8.1 years, 3.2% (112/3,515) opted for BPM. The uptake of BPM was largely dependent on lifetime risk estimates of developing breast cancer using the Tyrer-Cuzick model: 1.8% of women at 25-32% lifetime risk, 2.5% of women at 33-39% lifetime risk, and 6.4% of women at 40-45% lifetime risk underwent BPM ($P < .005$). We found an uptake rate of BPM of 5.7% (16/283) in women with no mutation identified.

Tamoxifen treatment for 5 years will reduce the incidence of estrogen receptor positive (but not estrogen receptor negative) breast cancer by 38%,[44] with the preventive effect being sustained for at least 5 years.[44] According to the IBIS-II trial, anastrozole reduces the incidence of breast cancer in high-risk postmenopausal women by 53% compared to placebo.[45] There have not been any primary prevention trials of tamoxifen conducted specifically among *BRCA1* or *BRCA2* mutation carriers, and only limited data from observational studies and secondary analyses are available.[46] Yet, as triple-negative cancers predominate in *BRCA1* mutation carriers, selective estrogen receptor modulators do not seem to substantially reduce the rate of breast cancers in this particular group.[47] Although guidelines recommend endocrine preventive therapy for high-risk women, the uptake of chemoprevention remains low, as reported by Skandarajah et al.[16] In our updated analysis, only 1.5% of *BRCA1* mutation carriers, 8.9% of *BRCA2* mutation carriers, and 7.4% of the noncarriers decided to take tamoxifen for medical prevention.

4.4 | Strengths, limitations, and future directions

This study is of a large number of women being managed in a uniform manner via a dedicated risk management clinic. We had comprehensive data available, and most of the noncarriers had their risk assessed using the BOADICEA program. Adherence to annual screening was high in our center, with less than 6% choosing to discontinue screening.

The small number of 22 breast cancer cases allowed for a descriptive analysis only. Also, the median follow-up time of 34 months is too short to assess the impact on mortality. Future analyses with a longer follow-up period will allow for a cost-effectiveness analysis of the current management practices at the BOCRMC that will not only account for the numbers of cancers detected within the clinic, but also for the number of women who benefited from the early breast cancer detection in terms of better survival.

Despite these limitations, our findings highlight the significant implications of centralizing the care of women at high risk for breast cancer with regard to tailor screening advice, early detection, and facilitate preventive strategies and provide up access to genetics services and research trials in women at high risk.

Cancers in high-risk noncarriers occur at ages that are similar to mutation carriers, and may require MRI to identify them. The multimodal surveillance program in the BOCRMC is effective in detecting early-stage breast cancers and the uptake of preventative strategies mirrors that of other studies.

The FRA-BOC tool provided by Cancer Australia is not optimal in assessing breast cancer risk. BOADICEA was introduced to further estimate the lifetime risks of breast cancer in noncarriers. However, a cutoff of 25% lifetime risk on BOADICEA would make us dismiss 77.6% of the noncarriers, even though a substantial amount of these women seems to be at high risk of developing breast cancer. Moreover, when compared to the FRA-BOC tool, there is a chance that BOADICEA would have defined other women to be at high risk for breast cancer. The introduction of SNP data and other risk factors is likely to improve the ability to identify those who benefit most from intensified surveillance. We need more follow-up data to determine an appropriate cutoff value on BOADICEA before refining referral criteria for noncarriers in Australia.

5 | CONCLUSIONS

Based on our findings, noncarriers at high risk for breast cancer should not be discharged from the BOCRMC. Further understanding of the apparent genetic components in noncarriers is imperative to standardize risk assessment and tailor recommendations for breast cancer screening and risk-reducing measures in *BRCA*-negative women. The growing number of GWAS may bring further insights into personalized risk assessment, ensuring that the limited resources available are used to maximum benefit.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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REFERENCES

1. Armstrong AC, Evans GD. Management of women at high risk of breast cancer. *BMJ*. 2014;348:g2756.
2. Ibarra JA. Pathology of BRCA tumors. In: Anees BC, ed. *Managing BRCA Mutation Carriers*. Cham, Switzerland: Springer International Publishing; 2017:89-117.
3. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. *Mol Oncol*. 2010;4(3):174-191.
4. Michailidou K, Lindstrom S, Dennis J, et al. Association analysis identifies 65 new breast cancer risk loci. *Nature*. 2017;551(7678):92-94.
5. Mavaddat N, Michailidou K, Dennis J, et al. Polygenic risk scores for prediction of breast cancer and breast cancer subtypes. *Am J Hum Genet*. 2018;104(1):21-34.
6. Kuchenbaecker KB, Hopper JL, Barnes DR, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *JAMA*. 2017;317(23):2402-2416.
7. Kotsopoulos J, Metcalfe K, Alston J, et al. Prospective study of high-risk, BRCA1/2-mutation negative women: the 'negative study'. *BMC Cancer*. 2014;14:221.
8. Cancer Australia. *Familial Risk Assessment--Breast and Ovarian Cancer (FRA-BOC)*. https://canceraustralia.gov.au/clinical-best-practice/gynaecological-cancers/familial-risk-assessment-fra-boc. Accessed April 22, 2019.
9. Schenberg T, Mitchell G, Taylor D, Saunders C. MRI screening for breast cancer in women at high risk; is the Australian breast MRI screening access program addressing the needs of women at high risk of breast cancer. *J Med Radiat Sci*. 2015;62(3):212-225.
10. Australian Government—Department of Health. *MRI (Magnetic Resonance Imaging) breast services Q&A (questions and answers)*. Canberra, Australia: Australian Government—Department of Health; 2013. https://www1.health.gov.au/internet/main/publishing.nsf/Content/mri-breast-services-q-and-a. Accessed April 22, 2019.
11. Antoniou AC, Cunningham AP, Peto J, et al. The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. *Br J Cancer*. 2008;98(8):1457-1466.
12. Rebbeck TR, Friebel T, Lynch HT, et al. Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. *J Clin Oncol*. 2004;22(6):1055-1062.
13. Phillips KA, Lindeman GJ. Breast cancer prevention for BRCA1 and BRCA2 mutation carriers: is there a role for tamoxifen. *Future Oncol*. 2014;10(4):499-502.
14. Lammert J, Grill S, Kiechle M. Modifiable lifestyle factors: opportunities for (hereditary) breast cancer prevention—a narrative review. *Breast Care*. 2018;13(2):109-114.
15. Kotsopoulos J. BRCA mutations and breast cancer prevention. *Cancers*. 2018;10(12):E524.
16. Skandarajah AR, Thomas S, Shackleton K, Chin-Lenn L, Lindeman GJ, Mann GB. Patient and medical barriers preclude uptake of tamoxifen preventative therapy in women with a strong family history. *Breast*. 2017;32:93-97.
17. ANZCTR. *BRCA-D, a pilot study evaluating a new breast cancer prevention strategy for BRCA1 and BRCA2 mutation carriers and high risk, non-BRCA carriers*. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12614000694617. Accessed April 22, 2019.
18. Kam JK, Naidu P, Rose AK, Mann GB. Five-year analysis of magnetic resonance imaging as a screening tool in women at hereditary risk of breast cancer. *J Med Imaging Radiat Oncol*. 2013;57(4):400-406.
19. Narod SA, Iqbal J, Giannakeas V, Sopik V, Sun P. Breast cancer mortality after a diagnosis of ductal carcinoma in situ. *JAMA Oncol*. 2015;1(7):888-896.
20. Husemann Y, Geigl JB, Schubert F, et al. Systemic spread is an early step in breast cancer. *Cancer Cell*. 2008;13(1):58-68.
21. Cancer Australia. *Breast cancer statistics: Breast cancer in Australia*. https://breast-cancer.canceraustralia.gov.au/statistics. Accessed April 22, 2019.
22. AIHW. *BreastScreen Australia monitoring report 2018*. https://www.aihw.gov.au/reports/cancer/breastscreen-australia-monitoring-report-2018/data. Accessed April 22, 2019.
23. Amir E, Freedman OC, Seruga B, Evans DG. Assessing women at high risk of breast cancer: a review of risk assessment models. *J Natl Cancer Inst*. 2010;102(10):680-691.
24. Ozanne EM, Drohan B, Bosinoff P, et al. Which risk model to use? Clinical implications of the ACS MRI screening guidelines. *Cancer Epidemiol Biomarkers Prev*. 2013;22(1):146-149.
25. Quante AS, Whittemore AS, Shriver T, Hopper JL, Strauch K, Terry MB. Practical problems with clinical guidelines for breast cancer prevention based on remaining lifetime risk. *J Natl Cancer Inst*. 2015;107(7):djv124.
26. MacInnis RJ, Bickerstaffe A, Apicella C, et al. Prospective validation of the breast cancer risk prediction model BOADICEA and a batch-mode version BOADICEACentre. *Br J Cancer*. 2013;109(5):1296-1301.
27. Park HL, Tran SM, Lee J, et al. Clinical implementation of a breast cancer risk assessment program in a multiethnic patient population: which risk model to use. *Breast J*. 2015;21(5):562-564.
28. Collins IM, Bickerstaffe A, Ranaweera T, et al. iPrevent(R): a tailored, web-based, decision support tool for breast cancer risk assessment and management. *Breast Cancer Res Treat*. 2016;156(1):171-182.

29. Lee A, Mavaddat N, Wilcox AN, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and non-genetic risk factors. *Genet Med*. 2019;21(8):1708-1718.
30. Monticciolo DL, Newell MS, Moy L, Niell B, Monsees B, Sickles EA. Breast cancer screening in women at higher-than-average risk: recommendations from the ACR. *J Am Coll Radiol*. 2018;15(3):408-414.
31. Phi X-A, Houssami N, Hooning MJ, et al. Accuracy of screening women at familial risk of breast cancer without a known gene mutation: individual patient data meta-analysis. *Eur J Cancer*. 2017;85:31-38.
32. Saadatmand S, Obdeijn IM, Rutgers EJ, et al. Survival benefit in women with BRCA1 mutation or familial risk in the MRI screening study (MRISC). *Int J Cancer*. 2015;137(7):1729-1738.
33. Evans DG, Harkness EF, Howell A, et al. Intensive breast screening in BRCA2 mutation carriers is associated with reduced breast cancer specific and all cause mortality. *Hered Cancer Clin Pract*. 2016;14:8.
34. Bick U, Engel C, Krug B, et al. High-risk breast cancer surveillance with MRI: 10-year experience from the German consortium for hereditary breast and ovarian cancer. *Breast Cancer Res Treat*. 2019;175(1):217-228.
35. Kuhl C, Weigel S, Schrading S, et al. Prospective multicenter cohort study to refine management recommendations for women at elevated familial risk of breast cancer: the EVA trial. *J Clin Oncol*. 2010;28(9):1450-1457.
36. Ingham SL, Sperrin M, Baildam A, et al. Risk-reducing surgery increases survival in BRCA1/2 mutation carriers unaffected at time of family referral. *Breast Cancer Res Treat*. 2013;142(3):611-618.
37. Giannakeas V, Narod SA. The expected benefit of preventive mastectomy on breast cancer incidence and mortality in BRCA mutation carriers, by age at mastectomy. *Breast Cancer Res Treat*. 2018;167(1):263-267.
38. Phillips KA, Jenkins MA, Lindeman GJ, et al. Risk-reducing surgery, screening and chemoprevention practices of BRCA1 and BRCA2 mutation carriers: a prospective cohort study. *Clin Genet*. 2006;70(3):198-206.
39. Metcalfe KA, Birenbaum-Carmeli D, Lubinski J, et al. International variation in rates of uptake of preventive options in BRCA1 and BRCA2 mutation carriers. *Int J Cancer*. 2008;122(9):2017-2022.
40. James PA, Mitchell G, Bogwitz M, Lindeman GJ. The Angelina Jolie effect. *Med J Aust*. 2013;199(10):646.
41. Evans DG, Wisely J, Clancy T, et al. Longer term effects of the Angelina Jolie effect: increased risk-reducing mastectomy rates in BRCA carriers and other high-risk women. *Breast Cancer Res*. 2015;17:143.
42. Liede A, Cai M, Crouter TF, Niepel D, Callaghan F, Evans DG. Risk-reducing mastectomy rates in the US: a closer examination of the Angelina Jolie effect. *Breast Cancer Res Treat*. 2018;171(2):435-442.
43. Evans DG, Lalloo F, Ashcroft L, et al. Uptake of risk-reducing surgery in unaffected women at high risk of breast and ovarian cancer is risk, age, and time dependent. *Cancer Epidemiol Biomarkers Prev*. 2009;18(8):2318-2324.
44. Cuzick J, Sestak I, Bonanni B, et al. Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of individual participant data. *Lancet*. 2013;381(9880):1827-1834.
45. Cuzick J, Sestak I, Forbes JF, et al. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomised placebo-controlled trial. *Lancet*. 2014;383(9922):1041-1048.
46. Paluch-Shimon S, Cardoso F, Sessa C, et al. Prevention and screening in BRCA mutation carriers and other breast/ovarian hereditary cancer syndromes: eSMO Clinical Practice Guidelines for cancer prevention and screening. *Ann Oncol*. 2016;27(suppl 5):v103-v110.
47. Phillips KA, Milne RL, Bassett JK, et al. Abstract P3-10-01: tamoxifen and contralateral breast cancer (CBC) risk for *BRCA1* and *BRCA2* mutation carriers: an updated analysis of data from the Kathleen Cuningham Foundation consortium for research into familial breast cancer, the International *BRCA1* and *BRCA2* Carrier cohort study and the breast cancer family registry. *Cancer Res*. 2018;78(4 Supplement):P3-10-01.

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APPENDIX

Addendum: Familial Risk Assessment—Breast and Ovarian Cancer (FRA-BOC)[7]

Category 1: At or slightly above average risk	Category 2: Moderately increased risk	Category 3: Potentially high risk
>95% of the female population	<4% of the female population	<1% of the female population
• No confirmed family history of breast cancer • One 1° relative diagnosed with breast cancer at age 50 or older • One 2° relative diagnosed with breast cancer at any age • Two 2° relatives on the same side of the family diagnosed with breast cancer at age 50 or older • Two 1° or 2° relatives diagnosed with breast cancer, at age 50 or older, but on different sides of the family (ie, one on each side of the family).	• One 1° relative diagnosed with breast cancer before the age of 50 (without the additional features of the potentially high-risk group—see category 3) • Two 1° relatives, on the same side of the family, diagnosed with breast cancer (without the additional features of the potentially high-risk group—see category 3) • Two 2° relatives, on the same side of the family, diagnosed with breast cancer, at least one before the age of 50, (without the additional features of the potentially high-risk group—see category 3).	• Women who are at potentially high risk of ovarian cancer • Two 1° or 2° relatives on one side of the family diagnosed with breast or ovarian cancer plus one or more of the following on the same side of the family: ○ Additional relative(s) with breast or ovarian cancer ○ Breast cancer diagnosed before The age of 40 ○ Bilateral breast cancer ○ Breast and ovarian cancer in the same woman ○ Jewish ancestry ○ Breast cancer in a male relative. • One 1° or 2° relative diagnosed with breast cancer at age 45 or younger plus another 1° or 2° relative on the same side of the family with sarcoma (bone/soft tissue) at age 45 or younger • Member of a family in which the presence of a high-risk breast cancer gene mutation has been established.
Risk of breast cancer up to age 75 is between 1 in 4 and 1 in 2. Risk may be more than three times the population average. Individual risk may be higher or lower if genetic test results are known.	Risk of breast cancer up to age 75 is between 1 in 8 and 1 in 4. This risk is 1.5 to three times the population average.	Risk of breast cancer up to age 75 is between 1 in 4 and 1 in 2. Risk may be more than three times the population average. Individual risk may be higher or lower if genetic test results are known.

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Modifiable Lifestyle Factors: Opportunities for (Hereditary) Breast Cancer Prevention – a Narrative Review

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Keywords

BRCA1 / *BRCA2* mutations · Breast cancer · Epidemiology · Health behavior · Hereditary breast cancer · Lifestyle · Nutrition · Obesity · Physical activity · Prevention

Summary

Increasing rates of obesity, lack of physical activity, sedentary behavior, and frequent alcohol consumption are major lifestyle-related risk factors for breast cancer. In fact, it has been estimated that about one-third of breast cancer cases are attributable to factors women can change. Most research has focused on examining the impact of one single exposure on breast cancer risk while adjusting for other risk modifiers. Capitalizing on big data, major efforts have been made to evaluate the combined impact of well-established lifestyle factors on overall breast cancer risk. At the individual level, data indicate that even simple behavior modifications could have a considerable impact on breast cancer prevention. Moreover, there is emerging new evidence that adopting a healthy lifestyle may be particularly relevant for women with hereditary susceptibility to breast cancer. On the absolute risk scale, studies suggest that the presence of certain risk factors, such as excessive body weight, had a substantially higher impact on breast cancer risk if women had a hereditary predisposition to cancer. The existing body of knowledge gives the medical professionals guidance as to which factors to focus on when counseling patients. However, well-designed randomized controlled trials utilizing objective methods are crucial to providing concrete recommendations.

Introduction

Four out of ten cancer cases among US women are believed to be preventable by healthier lifestyle choices [1]. In particular, excessive body weight, physical inactivity, frequent alcohol consumption, and the increased availability of calorie-dense foods are adding to the growing breast cancer burden. Furthermore, these unhealthy lifestyle choices threaten to offset the steady decline in cancer mortality [2]. Yet, the American Society of Clinical Oncology identified serious gaps in public knowledge about lifestyle factors contributing to cancer risk: Despite obesity being a leading preventable cause of cancer, only 31% of the people surveyed recognized the link [3]. Major public health efforts are necessary to improve public awareness and to incorporate lifestyle recommendations into clinical practice which can be applied both at the individual and the collective level. Here, we review the emerging evidence on modifiable lifestyle factors across the lifespan that offer an opportunity for breast cancer prevention by means of healthier living – even in women with genetic susceptibility to breast cancer.

Studies considered for this review were obtained from PubMed searches based on the search terms breast cancer, hereditary breast cancer, *BRCA*, risk factor, lifestyle, weight, obesity, physical activity, exercise, sedentariness, diet, and smoking. We critically reviewed references of all relevant publications (including review articles) to identify additional articles. Only studies published after 2002 that evaluated breast cancer risk were included. Studies examining the impact of lifestyle factors on clinical outcomes among breast cancer survivors were excluded.

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Excessive Body Weight, Distribution of Fat Mass, Insulin Signaling, and Breast Cancer Risk

A large body of research points to the fact that obesity increases a person's risk for at least 13 different types of cancer [4, 5], including postmenopausal breast cancer. The association between body weight and breast cancer risk varies by menopausal status: Evidence suggests that a high body mass index (BMI) may be associated with a decrease in premenopausal breast cancer risk, but is strongly associated with an increased risk of developing postmenopausal breast cancer [6]. A meta-analysis of cohort studies showed an 18% decreased risk of premenopausal breast cancer per 5 kg/m² increase in BMI during young adulthood [7]. In the Nurses' Health Study II, women indicating a BMI of ≥27.5 kg/m² at age 18 years had a 39% decrease in premenopausal breast cancer risk compared to their lean counterparts (BMI of 20.0–22.4 kg/m²) [8]. The reduction in premenopausal breast cancer risk with increasing BMI during the teen years has been attributed to irregular menstrual cycles and ovulatory infertility. Yet, after adjusting for these factors in the Nurses' Health Study II, they showed only little impact on the association [8]. Research suggests that body fatness at young ages may be associated with slower adolescent growth and lower levels of both progesterone and insulin-like growth factor 1 (IGF-1) [7, 9]. Still, the underlying biological mechanisms are not well-delineated and warrant further evaluation. Examining the role of obesity in premenopausal breast cancer in terms of specific tumor subtypes, Pierobon and Frankenfeld [10] observed a positive association between obesity and triple-negative breast cancer among premenopausal women, reporting an increase in risk of 43% (95% confidence interval (CI) 1.23–1.65).

Notably, adipose tissue is a major source of estrogen synthesis in postmenopausal women, an established risk factor in breast carcinogenesis, and evidence supports a clear association between body fatness and a substantially increased risk of postmenopausal breast cancer [11]. In a meta-analysis of prospective studies examining the association between BMI and postmenopausal breast cancer risk, Renehan et al. [12] found a 12% increase in risk per 5 kg/m² increase in BMI. Carpenter et al. [13] provided evidence that the positive association between BMI and postmenopausal breast cancer risk may be modified by family history of breast cancer: Postmenopausal women who had at least 1 first-degree relative with breast cancer and who had a current BMI of ≥27.1 kg/m² were at a 2.9 times greater breast cancer risk than women with a positive family history whose current BMI was <21.7 kg/m² (95% CI 1.86–4.54).

Several studies examined the association between weight change throughout the women's lifespan, i.e., mostly weight gained from early adulthood to present, and subsequent breast cancer risk [11]. A key limitation of this measure is that it may miss any weight loss at varying time points in a woman's lifespan. Eliassen et al. [14] showed that a weight gain of at least 25 kg since age 18 years elevated postmenopausal breast cancer risk by 45% (95% CI 1.3–1.7, p-trend < 0.001) compared to women whose weight remained stable (weight fluctuations of ≤2 kg). A weight gain of at least 10 kg following menopause conferred an 18% increased risk of breast cancer compared to a stable weight at the same period of time (95% CI 1.0–1.4, p-trend = 0.002) [14]. Based on data from the Women's Health Initiative observational study, Chlebowski et al. [15] demonstrated that a modest weight loss after menopause, i.e., a relative weight loss of at least 5% of one's body weight, could lower breast cancer risk by 12% relative to stable weight (95% CI 0.78–0.98).

In addition to a higher risk of developing breast cancer, overweight women tend to present with larger tumors at the time of diagnosis when compared with their normal-weight counterparts [16].

Case-control studies suggest that a woman's body fat distribution may influence the hormone receptor status of the breast cancer she is susceptible to [17]. Both general and central obesity have been associated with greater breast cancer risk [11]. Obese women with larger amounts of subcutaneous fat, as measured by BMI, may have a significantly higher risk of developing hormone receptor-positive breast cancer. In contrast, larger amounts of visceral fat, indicated by a high waist-to-hip ratio, may be associated with a greater risk of developing hormone receptor-negative breast cancer, independent of BMI [17].

As scientists continue to explore the relationship between obesity and cancer, there is rapidly increasing interest in the insulin signaling pathway [18]. Based on data from the Women's Health Initiative Study, Gunter et al. [19] conducted a case-control analysis to prospectively examine the incidence of postmenopausal breast cancer among nondiabetic women. Their findings suggest that insulin levels were positively associated with breast cancer risk (hazard ratio (HR) 1.46; 95% CI 1.00–2.13; p-trend = 0.02), confirming that hyperinsulinemia is an independent risk factor for postmenopausal breast cancer. While experimental evidence supports a synergistic interaction between estrogen receptor activation and increased IGF-1 signaling with regard to breast carcinogenesis, the data from the prospective European Prospective Investigation into Cancer and Nutrition (EPIC) cohort indicate that higher circulating IGF-1 levels may increase the risk of hormone receptor-positive breast cancer diagnosed after age 50 years (odds ratio (OR) 1.38, 95% CI 1.01–1.89; p-trend = 0.01) [20].

Physical Activity and Breast Cancer Risk

Numerous scientific studies have demonstrated a protective role of physical activity in breast cancer etiology, and anthropometric factors do not attenuate this association [11]. Collectively, the evidence supports an inverse association between physical activity and breast cancer, with a risk reduction of 20–30% when comparing the most physically active to the least active women [21, 22] and depending on the study design, population studied, and level of physical activity. The relationship exists for both pre- and postmenopausal women, with greater risk reductions observed among postmenopausal women [7, 21]. The magnitude of risk reduction appears to be stronger for strenuous than for moderate levels of exercise [21, 23].

Both retrospective and prospective studies confirmed a dose-response relationship between increasing levels of physical activity and breast cancer risk, and the association was true for all pathological subtypes of cancer [11, 24]. In the Nurses' Health Study II, adolescent physical activity from ages 14–17 years was inversely associated with premenopausal breast cancer risk, with a risk reduction of 19% (95% CI 0.69–0.95) [25].

Even though research provides consistent findings linking physical activity to breast cancer, a big limitation of these studies is the reliance on women's self-reports instead of objectively measured data. To date, only one research group used accelerometer data to evaluate the association between physical activity level and breast cancer incidence [6]. Among 2,160 Polish women, Dallal et al. [26] reported a 61% decrease in risk for women in the highest quartile of moderate-to-vigorous accelerometer-based measures of physical activity compared to women in the lowest quartile (95% CI 0.27–0.56; p-trend < 0.0001).

Sedentariness and Breast Cancer Risk

Long amounts of time spent sitting have been shown to influence breast cancer risk, and the positive association seems to be independent of physical activity. Based on accelerometer data and after adjustment for physical activity, Dallal et al. [26] found an 81% increased risk of breast cancer in women with the longest sedentary time compared to women with the shortest duration of sitting (95% CI 1.26–2.60; p-trend = 0.001). With regard to occupational sedentariness, Johnsson et al. [27] observed a 20% increased risk of breast cancer diagnosed before age 55 years (95% CI 1.05–1.37) compared to women with less sedentary occupations. The results were replicated in a study of African-Americans evaluating the association between total time spent sitting and subsequent breast cancer risk [28].

Diet and Breast Cancer Risk

In separate studies, alcohol consumption emerged as the strongest and most consistent dietary factor linked with breast cancer. In 2017, the World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) published a dose-response meta-analysis for premenopausal breast cancer which revealed a 5% increased risk per 10 g of ethanol consumed per day (95% CI 1.02–1.08) [7]. For postmenopausal breast cancer, the researchers observed a 9% increased risk per 10 g of ethanol consumed per day (95% CI 1.07–1.12) [7]. Findings indicate that frequent alcohol consumption may put a woman with at least 1 breast cancer-affected first-degree relative at greater risk for breast cancer than women with no family history [29].

Case-control studies suggest that balanced diets, consisting of substantial amounts of wholegrain, fiber, fruits, and vegetables, are associated with reduced breast cancer risk, especially when adopted early in life, i.e., during childhood [6]. Based on the Nurses' Health Study II, Harris et al. [30] reported that an adolescent and early adulthood dietary pattern characterized by sugar-sweetened soft drinks, refined grains, red and processed meat, and margarine, and low intake of leafy vegetables and cruciferous vegetables was associated with an increased incidence of premenopausal breast cancer (HR 1.35; 95% CI 1.06–1.73; p-trend = 0.002 for adolescent diet, and HR 1.41, 95% CI 1.11–1.78; p-trend = 0.006 for early adulthood diet) [30]. In 2017, the WCRF/AICR indicated that only limited evidence exists for a decrease in breast cancer risk associated with the consumption of foods containing carotenoids or the consumption of non-starchy vegetables, respectively [7]. In fact, randomized controlled trials for studying diet-cancer relationships have failed to demonstrate a significant impact of diet on breast cancer risk. Only limited and non-significant trends exist for an association between a low-fat dietary pattern and reduced breast cancer risk [7, 11]. The EPIC study investigated the association between the adherence to the Mediterranean diet and risk of breast cancer among 335,062 European women, with an average follow-up of 11 years. The data indicate that adherence to the Mediterranean diet excluding alcohol was associated with a decrease in risk for postmenopausal breast cancer (HR 0.93; 95% CI 0.87–0.99; p-trend = 0.037), particularly in the case of hormone receptor-negative tumors (HR 0.80; 95% CI 0.65–0.99; p-trend = 0.043). The PREDIMED trial was the first randomized controlled trial to support these findings, providing additional evidence for a protective effect of a Mediterranean diet, supplemented by extra virgin olive oil, in breast cancer, with a decrease in risk of 68% (95% CI 0.13–0.79) [31]. Well-designed prospective studies focusing on diet patterns rather than dietary components are warranted to address the critical gap in the current literature regarding the role of diet in breast cancer risk.

Smoking and Breast Cancer Risk

The relationship between smoking and breast cancer has been studied extensively; however, findings have been inconclusive [11]. Emerging evidence derived from better-designed epidemiologic studies suggests a positive association between breast cancer and tobacco consumption in populations with high smoking prevalence, higher pack-years, and long durations of smoking [11, 32–34]. In the largest cohort examined, Dossus et al. [33] provide evidence that both active and passive smoking contribute to a substantially increased risk of breast cancer, particularly for increasing pack-years of active smoking between menarche and first full-term pregnancy (HR 1.73, 95% CI 1.29–2.32 for every increase of 20 pack-years).

Joint Impact of Lifestyle Factors on Breast Cancer Risk

Since cancer predisposition is multifactorial in origin, caused by a complex interplay between genetic factors and a multitude of

non-genetic exposures such as environmental influences, reproductive and lifestyle factors – many of them occurring concomitantly, modifiable risk assessment cannot simply be reduced to a single hypothetical factor. Failure to include relevant exposures most possibly results in decreased power and biased risk estimates, whereas considering a large amount of potential influences may lead to challenges in both statistical implementation and interpretation, particularly in correlated factors. Further, both preventive and harmful lifestyle behaviors tend to appear in clusters. Recently, major efforts have been made to evaluate the combined impact of selected lifestyle factors on breast cancer risk. Based on data from the EPIC study, McKenzie et al. [35] evaluated a healthy lifestyle index score (HLIS) to investigate the joint effect of 5 modifiable lifestyle factors on postmenopausal breast cancer risk. The HLIS is composed of diet, physical activity, smoking, alcohol consumption, and anthropometric factors, with higher values indicating healthier behaviors. With each point added to a person's HLIS, postmenopausal breast cancer risk decreased by 3%, suggesting that an overall healthy lifestyle may substantially lower the risk of developing postmenopausal breast cancer. Ellingjord-Dale et al. [36] replicated these findings in a Norwegian cohort. Risky lifestyle behaviors were defined as follows: ever-smoking, weekly consumption of >2 glasses of alcoholic beverage, <3 h leisure time physical activity weekly, ever-use of menopausal hormone therapy, and BMI > 25 kg/m^2. There was a linear dose-response relationship between the number of risky lifestyle behaviors and hormone receptor-positive breast cancer: Women who had 5 risky lifestyle behaviors were at a 2.38 times greater risk of luminal A-like breast cancer compared to women with no risky lifestyle behaviors (95% CI 1.58–3.59; p-trend < 0.0001). Taken together, these findings show preliminary evidence that an overall healthy lifestyle may contribute to a sizeable decrease in breast cancer risk.

Impact of Modifiable Risk Factors on *BRCA*-Associated Breast Cancer

Women who inherit a deleterious *BRCA1* or *BRCA2* mutation face a high lifetime risk of developing breast cancer, between 69 and 72% [37] compared to 12% in the general population [38]. Among *BRCA* mutation carriers, primary prevention of breast cancer is limited to prophylactic bilateral mastectomy; however, mutation carriers frequently inquire about less invasive prevention options [39]. Both the incomplete penetrance and the regional differences in penetrance of an inherited *BRCA1* or *BRCA2* mutation suggest that environmental exposures may influence risk [40]. While various reproductive and hormonal factors have shown to impact *BRCA*-associated cancer risk [41], suggestive evidence exists that lifestyle factors, including body weight, adolescent physical activity, calorie restriction, and non-smoking, may contribute to a decrease in the number of *BRCA*-associated breast cancer cases [42, 43].

In an early study, King et al. [44] reported that the risk for early-onset breast cancer was lower if gene mutation carriers were born before 1940, had given birth, had a healthy weight at menarche and age 21, and were physically active during adolescence. Kotsopoulos et al. [45] showed that a weight loss of at least 10 pounds between ages 18 and 30 was associated with a 53% decreased risk of *BRCA*-associated breast cancer between ages 30 and 49 (95% CI 0.28–0.79). Pijpe et al. [46] demonstrated a significant 42% reduction in risk (95% CI 0.35–0.94; p-trend = 0.05) with increasing levels of physical activity prior to, but not after, age 30. In addition to mechanistic data linking physical activity to reductions in endogenous sex hormone levels, lower IGF-1 levels, and an improved immune function [22, 47], gene-environment interactions are of increasing research interest with regard to *BRCA*-related tumors. Using an in vivo model, Wang et al. [48] demonstrated that prepubertal physical activity was associated with a significant increase in *BRCA1*, *p53*, estrogen receptor *(ER)-α*, and *ER-β* mRNA expression in mammary glands of adult rats versus control (unexercised) rats ($p < 0.03$). We recently reported that, even in a small study cohort of 68 *BRCA* mutation carriers, study participants indicating a higher level of physical activity during adolescence had a significantly lower cancer prevalence ($p = 0.019$) [49]. Additionally, we observed a significantly higher cancer prevalence in smokers compared to non-smokers ($p < 0.001$) [49]. Pettapiece-Phillips et al. [50] showed that uninterrupted sedentary behavior was associated with decreased *BRCA1* mRNA expression ($p = 0.02$). Whether this finding translates into a potentially harmful effect with regard to *BRCA*-associated cancer risk is under active study. Nkondjock et al. [51] reported a positive association between total energy intake and *BRCA*-associated breast cancer risk when comparing the highest tertile of calorie intake with the lowest tertile (OR 2.76, 95% CI 1.10–7.02; p-trend = 0.026). In another analysis, Nkondjock and Ghadirian [52] demonstrated that a balanced diet of high quality was associated with a 65–82% decreased risk of *BRCA*-related breast cancer (OR 0.35, 95% CI 0.12–1.02; p-trend = 0.034 for Diet Quality Index-Revised (DQI-R) and OR 0.18, 95% CI 0.05–0.68; p-trend = 0.006 for Canadian Healthy Eating Index (CHEI)). However, analyses were all limited by a retrospective study design with small sample sizes, providing significant potential for biases.

Trygvadottir et al. [53] investigated changes in penetrance over time with regard to the cumulative breast cancer incidence before age 70 years in Icelandic women. The investigators found a 4-fold increase in the cumulative incidence of breast cancer between 1920 and 2000 among *BRCA2* mutation carriers (from 18.6 to 71.9%) and women in the general population (from 1.8 to 7.5%). Indeed, on the absolute risk scale, the adoption of a healthy lifestyle will affect breast cancer risk to a considerably greater extent among women with a hereditary susceptibility to breast cancer compared to women without a family history of cancer. Quante et al. [54] evaluated the performance of the IBIS Breast Cancer Risk Evaluation Tool, with and without accounting for BMI, for predicting breast cancer occurrence in postmenopausal women. The authors found a significant absolute BMI-induced difference in IBIS-assigned 10-year risk, comparing women with a BMI of 27 kg/m^2 to women with a BMI of 21 kg/m^2 with regards to hereditary predisposition to cancer: The absolute BMI-induced difference in the

model-assigned 10-year risk ranged from 0.3% for women with neither affected first-degree relatives nor a *BRCA1* mutation to 4.5% for mutation carriers with 3 breast cancer-affected relatives. Additionally, at the individual level, Quante et al. [54] demonstrated that overweight women who had originally been classified as 'high risk' patients for developing breast cancer could be reclassified as 'low risk' only by reducing their BMI.

Among *BRCA* mutation carriers, two independent studies demonstrated that the receipt of a positive *BRCA1/2* genetic test result contributed to significant lifestyle changes [55, 56]. We recently reported that women with hereditary *BRCA* mutations tend to adopt healthier lifestyles compared to women from the general population [49]. However, radical changes in health behavior could potentially result in the uptake of harmful behaviors. For instance, given the widespread use of folic acid supplementation, research efforts have been made to elucidate the relationship between folate status and breast cancer risk. Even though the role of folate in the development of *BRCA*-related breast cancer is not clear, Kim et al. [57] indicated that elevated plasma folate concentrations might be associated with an increased risk for breast cancer among *BRCA* mutation carriers.

A prospective evaluation of multiple lifestyle behaviors, collected at various time points, with the utilization of objective methods to capture body size, physical activity, and dietary habits, is crucial to provide evidence-based, safe, and effective strategies for this high-risk group. Moreover, providing measurable data is necessary to facilitate the counselees' understanding of both non-modifiable and modifiable risk information [58].

Aiming to elucidate the impact of non-genetic modifiers on *BRCA*-associated breast cancer risk, the LIBRE study is the first prospective randomized lifestyle intervention trial worldwide involving cancer-affected and -unaffected *BRCA* mutation carriers [59, 60]. The purpose of the randomized, 2-armed (1:1), multicenter, interdisciplinary, prospective, and open study is to demonstrate that a structured intervention program, consisting of endurance training paired with the Mediterranean diet, will improve BMI, physical fitness, and adherence to the Mediterranean diet pattern. The long-term goals of the trial are to demonstrate a decrease in breast cancer risk, an inhibited progression of disease, and a reduced cancer mortality rate in *BRCA* mutation carriers following a healthy lifestyle. Ultimately, by utilizing a variety of objective methods and by analyzing the joint effects of modifiable lifestyle factors, the LIBRE trial aims to provide data on lifestyle options of preventive value that could be translated into the practice of genetic counseling.

Conclusion

Building upon the growing evidence linking lifestyle factors to (*BRCA*-associated) breast cancer, we suggest that action should be taken to incorporate timely lifestyle recommendations into the daily practice of clinicians and genetic counselors. To begin with, maintaining a healthy weight, limiting alcohol consumption, smoking cessation, and being physically active on a regular basis is a message medical practitioners and patients can act upon until more precise recommendations can be made.

Disclosure Statement

The authors declare that they have no conflict of interest.

References

1 Islami F, Goding Sauer A, Miller KD, et al: Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. CA Cancer J Clin 2018;68:31–54.
2 Ma J, Ward EM, Siegel RL, Jemal A: Temporal trends in mortality in the United States, 1969-2013. JAMA 2015;314:1731–1739.
3 ASCO: National Cancer Opinion Survey, 2017. *www.asco.org/research-progress/reports-studies/national-cancer-opinion-survey*.
4 Massetti GM, Dietz WH, Richardson LC: Excessive weight gain, obesity, and cancer: opportunities for clinical intervention. JAMA 2017;318:1975–1976.
5 Pearson-Stuttard J, Zhou B, Kontis V, Bentham J, Gunter MJ, Ezzati M: Worldwide burden of cancer attributable to diabetes and high body-mass index: a comparative risk assessment. Lancet Diabetes Endocrinol 2018;6:95–104.
6 Kerr J, Anderson C, Lippman SM: Physical activity, sedentary behaviour, diet, and cancer: an update and emerging new evidence. Lancet Oncol 2017;18:e457–471.
7 World Cancer Research Fund International/AICR: Continuous Update Project Report: Diet, Nutrition, Physical Activity and Breast Cancer. 2017. *wcrf.org/breast-cancer-2017. All CUP reports are available at wcrf.org/cupreports*.
8 Michels KB, Terry KL, Willett WC: Longitudinal study on the role of body size in premenopausal breast cancer. Arch Intern Med 2006;166:2395–2402.
9 Dowsett M, Folkerd E: Reduced progesterone levels explain the reduced risk of breast cancer in obese premenopausal women: a new hypothesis. Breast Cancer Res Treat 2015;149:1–4.
10 Pierobon M, Frankenfeld CL: Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis. Breast Cancer Res Treat 2013;137:307–314.
11 Li C (ed): Breast Cancer Epidemiology. New York, Springer, 2010.
12 Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M: Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008;371:569–578.
13 Carpenter CL, Ross RK, Paganini-Hill A, Bernstein L: Effect of family history, obesity and exercise on breast cancer risk among postmenopausal women. Int J Cancer 2003;106:96–102.
14 Eliassen AH, Colditz GA, Rosner B, Willett WC, Hankinson SE: Adult weight change and risk of postmenopausal breast cancer. JAMA 2006;296:193–201.
15 Chlebowski RT LJ, Anderson GL, Simon M, et al: Weight change in postmenopausal women and breast cancer risk in the Women's Health Initiative Observational Study. GS5-07, San Antonio Breast Cancer Symposium, San Antonio, TX, Dec 5–9, 2017.
16 Strand F, Humphreys K, Holm J, et al: Large breast cancers in women attending regular screening: risk factors and implications for prognosis. Ann Meet Radiol Soc N Am; Nov 26–Dec 1, 2017, Chicago, IL.
17 Wang F, Liu L, Cui S, et al: Distinct effects of body mass index and waist/hip ratio on risk of breast cancer by joint estrogen and progestogen receptor status: results from a case-control study in Northern and Eastern China and implications for chemoprevention. Oncologist 2017;22:1431–1443.
18 Bjorner S, Rosendahl AH, Simonsson M, et al: Body mass index influences the prognostic impact of combined nuclear insulin receptor and estrogen receptor expression in primary breast cancer. Front Endocrinol (Lausanne) 2017;8:332.
19 Gunter MJ, Hoover DR, Yu H, et al: Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2009;101:48–60.
20 Kaaks R, Johnson T, Tikk K, et al: Insulin-like growth factor I and risk of breast cancer by age and hormone receptor status – a prospective study within the EPIC cohort. Int J Cancer 2014;134:2683–2690.

21 Neilson HK, Farris MS, Stone CR, Vaska MM, Brenner DR, Friedenreich CM: Moderate-vigorous recreational physical activity and breast cancer risk, stratified by menopause status: a systematic review and meta-analysis. Menopause 2017;24:322–344.
22 Friedenreich CM: Physical activity and breast cancer: review of the epidemiologic evidence and biologic mechanisms. Recent Results Cancer Res 2011;188: 125–139.
23 Friedenreich CM, Cust AE: Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects. Br J Sports Med 2008;42:636–647.
24 Lope V, Martin M, Castello A, et al: Physical activity and breast cancer risk by pathological subtype. Gynecol Oncol 2017;144:577–585.
25 Boeke CE, Eliassen AH, Oh H, Spiegelman D, Willett WC, Tamimi RM: Adolescent physical activity in relation to breast cancer risk. Breast Cancer Res Treat 2014;145:715–724.
26 Dallal CM, Brinton LA, Matthews CE, et al: Accelerometer-based measures of active and sedentary behavior in relation to breast cancer risk. Breast Cancer Res Treat 2012;134:1279–1290.
27 Johnsson A, Broberg P, Johnsson A, Tornberg AB, Olsson H: Occupational sedentariness and breast cancer risk. Acta Oncol 2017;56:75–80.
28 Nomura SJ, Dash C, Rosenberg L, Palmer J, Adams-Campbell LL: Sedentary time and breast cancer incidence in African American women. Cancer Causes Control 2016;27:1239–1252.
29 Scoccianti C, Lauby-Secretan B, Bello PY, Chajes V, Romieu I: Female breast cancer and alcohol consumption: a review of the literature. Am J Prev Med 2014; 46(3 suppl 1):S16–25.
30 Harris HR, Willett WC, Vaidya RL, Michels KB: An adolescent and early adulthood dietary pattern associated with inflammation and the incidence of breast cancer. Cancer Res 2017;77:1179–1187.
31 Toledo E, Salas-Salvado J, Donat-Vargas C, et al: Mediterranean diet and invasive breast cancer risk among women at high cardiovascular risk in the PREDIMED trial: a randomized clinical trial. JAMA Intern Med 2015;175:1752–1760.
32 Terry PD, Miller AB, Rohan TE: Cigarette smoking and breast cancer risk: a long latency period? Int J Cancer 2002;100:723–728.
33 Dossus L, Boutron-Ruault MC, Kaaks R, et al: Active and passive cigarette smoking and breast cancer risk: results from the EPIC cohort. Int J Cancer 2014;134: 1871–1888.
34 Glantz SA, Johnson KC: The surgeon general report on smoking and health 50 years later: breast cancer and the cost of increasing caution. Cancer Epidemiol Biomarkers Prev 2014;23:37–46.
35 McKenzie F, Ferrari P, Freisling H, et al: Healthy lifestyle and risk of breast cancer among postmenopausal women in the European Prospective Investigation into Cancer and Nutrition cohort study. Int J Cancer 2015; 136:2640–2648.
36 Ellingjord-Dale M, Vos L, Hjerkind KV, et al: Alcohol, physical activity, smoking, and breast cancer subtypes in a large, nested case-control study from the Norwegian Breast Cancer Screening Program. Cancer Epidemiol Biomarkers Prev 2017;26:1736–1744.
37 Kuchenbaecker KB, Hopper JL, Barnes DR, et al: Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. JAMA 2017; 317:2402–2416.
38 Howlader N, Noone AM, Krapcho M, et al: SEER Cancer Statistics Review, 1975–2013. Bethesda, National Cancer Institute, 2016.
39 Liede A, Mansfield CA, Metcalfe KA, et al: Preferences for breast cancer risk reduction among BRCA1/BRCA2 mutation carriers: a discrete-choice experiment. Breast Cancer Res Treat 2017;165:433–444.
40 Lubinski J, Huzarski T, Byrski T, et al: The risk of breast cancer in women with a BRCA1 mutation from North America and Poland. Int J Cancer 2012;131: 229–234.
41 Narod SA: BRCA mutations in the management of breast cancer: the state of the art. Nat Rev Clin Oncol 2010;7:702–707.
42 Friebel TM, Domchek SM, Rebbeck TR: Modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: systematic review and meta-analysis. J Natl Cancer Inst 2014;106:dju091.
43 Pettapiece-Phillips R, Narod SA, Kotsopoulos J: The role of body size and physical activity on the risk of breast cancer in BRCA mutation carriers. Cancer Causes Control 2015;26:333–344.
44 King MC, Marks JH, Mandell JB; New York Breast Cancer Study Group: Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643–646.
45 Kotsopoulos J, Olopado OI, Ghadirian P, et al: Changes in body weight and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res 2005;7:R833–843.
46 Pijpe A, Manders P, Brohet RM, et al: Physical activity and the risk of breast cancer in BRCA1/2 mutation carriers. Breast Cancer Res Treat 2010;120:235–244.
47 Neilson HK, Conroy SM, Friedenreich CM: The influence of energetic factors on biomarkers of postmenopausal breast cancer risk. Curr Nutr Rep 2014;3:22–34.
48 Wang M, Yu B, Westerlind K, et al: Prepubertal physical activity up-regulates estrogen receptor beta, BRCA1 and p53 mRNA expression in the rat mammary gland. Breast Cancer Res Treat 2009;115:213–220.
49 Grill S, Yahiaoui-Doktor M, Dukatz R, et al: Smoking and physical inactivity increase cancer prevalence in BRCA-1 and BRCA-2 mutation carriers: results from a retrospective observational analysis. Arch Gynecol Obstet 2017;296:1135–1144.
50 Pettapiece-Phillips R, Kotlyar M, Chehade R, et al: Uninterrupted sedentary behavior downregulates BRCA1 gene expression. Cancer Prev Res (Phila) 2016;9:83–88.
51 Nkondjock A, Robidoux A, Paredes Y, Narod SA, Ghadirian P: Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. Breast Cancer Res Treat 2006;98:285–294.
52 Nkondjock A, Ghadirian P: Diet quality and BRCA-associated breast cancer risk. Breast Cancer Res Treat 2007;103:361–369.
53 Tryggvadottir L, Sigvaldason H, Olafsdottir GH, et al: Population-based study of changing breast cancer risk in Icelandic BRCA2 mutation carriers, 1920–2000. J Natl Cancer Inst 2006;98:116–122.
54 Quante AS, Herz J, Whittemore AS, Fischer C, Strauch K, Terry MB: Assessing absolute changes in breast cancer risk due to modifiable risk factors. Breast Cancer Res Treat 2015;152:193–197.
55 Digianni LM, Rue M, Emmons K, Garber JE: Complementary medicine use before and 1 year following genetic testing for BRCA1/2 mutations. Cancer Epidemiol Biomarkers Prev 2006;15:70–75.
56 Spector D: Lifestyle behaviors in women with a BRCA1 or BRCA2 genetic mutation: an exploratory study guided by concepts derived from the Health Belief Model. Cancer Nurs 2007;30:E1–10.
57 Kim SJ, Zuchniak A, Sohn KJ, et al: Plasma folate, vitamin B-6, and vitamin B-12 and breast cancer risk in BRCA1- and BRCA2-mutation carriers: a prospective study. Am J Clin Nutr 2016;104:671–677.
58 Wright CE, Harvie M, Howell A, Evans DG, Hulbert-Williams N, Donnelly LS: Beliefs about weight and breast cancer: an interview study with high risk women following a 12 month weight loss intervention. Hered Cancer Clin Pract 2015;13:1.
59 Kiechle M, Engel C, Berling A, et al: Effects of lifestyle intervention in BRCA1/2 mutation carriers on nutrition, BMI, and physical fitness (LIBRE study): study protocol for a randomized controlled trial. Trials 2016; 17:368.
60 Kiechle M, Dukatz R, Yahiaoui-Doktor M, et al: Feasibility of structured endurance training and Mediterranean diet in BRCA1 and BRCA2 mutation carriers – an interventional randomized controlled multicenter trial (LIBRE-1). BMC Cancer 2017;17:752.