

**A Literature Review of Risk Factors for the Development of Breast Cancer:
Risks and Prevention Methods**

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Introduction

In general, cancer arises from an accumulation of mutations in genes that control cell growth and cell cycle progression, leading to unregulated proliferation of cells. While cell proliferation itself can be relatively benign, such cells often cannot adequately undergo mitotic checkpoints for DNA repair, leading to genetic instability and subsequent mutations that may promote invasion of these cells to adjacent tissues, thereby creating cancer. Therefore, many of the pathways that increase cancer risk promote cell proliferation or cause instability of the genome (Seo et al., 2012). When such pathways are promoted in breast tissue, an increase in breast cancer risk may occur (Nandi et al., 1995).

Breast cancer, as the name suggests, is the formation of a malignant tumor in one or both breasts. Breast cancer is a prominent health concern among women worldwide. Breast cancer can also occur in men, but it is 100 times more common in women (What are the risk factors, 2013). Currently, there are over 2.9 million breast cancer survivors in the United States alone. In 2013, it is estimated that there will be 39,620 deaths due to breast cancer in the U.S. (What are the key statistics, 2013). Other than non-melanoma skin cancer, breast cancer is the most common cancer diagnosed in women worldwide; it is also responsible for more female deaths than any other type of cancer (Boyle and Levin, 2008). In the U.S., approximately one out of every eight women or about 12.4% of the female population will be diagnosed with breast cancer. Therefore, the average woman living in the U. S. has about a 12.4% risk of developing breast cancer. Anything that increases this risk is termed a “risk factor” (Howlader et al., 2012). Established risk factors account for about 30% of breast cancer cases; however, identifying the main cause of breast cancer in any individual is challenging because there are typically many

potentially influential elements to consider (Madigan et al, 1995). Risk factors are sometimes outside of one's control, such as genetics. However, other risk factors, like alcohol use or obesity, are generally preventable. Therefore, knowledge of how a certain risk factor affects an individual's breast cancer risk, as well as potential ways to avoid or reduce the risk associated with such factors, may help a woman assess her risk.

A. Inherited mutations in *BRCA1* and *BRCA2*

Inherited mutations in *BRCA1* and *BRCA2* are potent risk factors for breast cancer

Since the discovery of *BRCA1* in 1990 and the discovery of *BRCA2* in 1994, a vast body of research has centered on discovering why mutations in these proteins significantly increase breast cancer risk (Narod et al., 2004). *BRCA1* and *BRCA2*, breast cancer susceptibility genes 1 and 2 that code for proteins *BRCA1* and *BRCA2* respectively, are genes that code for tumor suppressor proteins (Gage et al., 2012). As such, they are important genetic factors in breast cancer risk. About 5-10% of breast cancer cases are characterized by a genetically inherited component (Gage et al., 2012). Approximately 80% of these cases are attributable to inherited mutations in *BRCA1* while another 15% are due to inherited mutations in *BRCA2* (Ford et al., 1998). These statistics mean that 3-5% of total breast cancer cases are attributable to mutations in *BRCA1* and/or *BRCA2* (Hereditary Breast and Ovarian Cancer, 2012). Although *BRCA1* and *BRCA2* mutations are present in only 1 in every 400 people, these mutations can increase one's risk of developing breast cancer by a factor of 10 to 20 (Foulkes, 2008 and Stratton, 2008). Therefore, an individual possessing a mutation in *BRCA1* has a 57% risk of developing breast cancer during their lifetime. Those with a mutation in *BRCA2* have a lifetime risk of 49% (Chen

et al., 2007). However, the risk for developing cancer may be as high as 80% for those with mutations in either gene, depending on the specific mutation present (What are the risk factors, 2013).

Like other inherited mutations, mutations in *BRCA1* and *BRCA2* are passed on as germline mutations, or mutations in gametes. An individual heterozygous for a mutation in *BRCA1* or *BRCA2* has a 50% chance of passing the mutated gene on to their offspring, regardless of their child's gender (Weitzel et al., 2007). Depending on the specific mutation, the protein that results from the mutated gene can lead to a defective BRCA1 or BRCA2 protein. To fully understand why defective BRCA1 and BRCA2 proteins lead to an increased risk for the development of breast cancer, the normal functions of the BRCA1 and BRCA2 proteins must be examined.

Healthy BRCA1 protein helps prevent the development of cancer

An expansive body of research indicates that BRCA1 plays a central role in DNA repair. A 1999 study found that a BRCA1 deficiency in embryonic mouse stem cells is associated with decreased ability to repair chromosomal double strand breaks that often occur as a result of normal metabolic processes. These results indicate that BRCA1 maintains genetic integrity, thereby helping to prevent the development of cancerous mutations (Moynahan et al., 1999). A later study revealed that BRCA1 functions as part of a larger complex of proteins in order to enact DNA repair. This complex is named BASC, BRCA1-associated genome surveillance complex. BASC is composed of at least 15 proteins including DNA damage sensors like ATM kinase, ATR kinase, and the RAD50-MRE11-NBS1 complex as well as mismatch repair proteins like the MSH2-MSH6 dimer and the MLH1-PMS2 dimer. BRCA1 associates with these proteins

in BASC and works synergistically with them in order to accomplish DNA repair (Wang et al., 2000).

More specifically, BRCA1 participates in a specific type of DNA repair known as homologous recombination (Moynahan et al., 1998). Homologous recombination ideally uses a sister chromatid, which is identical to the damaged chromatid, as a repair template in order to mend a chromosomal double strand break (Johnson et al., 1999). Nonhomologous end joining, an alternate DNA repair pathway to homologous recombination, does not involve use of a homologous template. As a result, this kind of DNA repair is more prone to inaccurate repair, which can contribute to genome instability. In vitro experiments have shown that BRCA1, in addition to controlling homologous recombination, may be linked to lower levels of nonhomologous end joining. Therefore, BRCA1 maintains genome integrity by both mediating accurate DNA repair and by possibly preventing faulty DNA repair in the form of nonhomologous end joining (Moynahan et al., 2012). Most cancer-inducing mutations in BRCA1 occur within its N-terminal RING domain, exons 11-13, and the BRCT domain (Clark et al., 2012). Examining the function of each of these protein regions helps illuminate the process by which BRCA1 mutations contribute to breast cancer risk.

Mutations in BRCA1 RING domain reduce ubiquitin ligase activity

The RING (Really Interesting Gene) domain contributes to BRCA1 function by inducing E3 ubiquitin ligase activity and promoting interactions with other proteins (Clark et al., 2012). The RING domain of BRCA1 is composed of a Zinc finger as well as two alpha helices (Brzovic et al., 2001). The two alpha helices are responsible for the association of BRCA1 with BARD1 (BRCA1 Associated RING Domain protein 1); the dimerization of BRCA1 and BARD1

proteins greatly increases BRCA1 E3 ubiquitin ligase activity. E3 ubiquitin ligase is necessary for ubiquitination of substrates to occur, and loss-of-function mutations in the RING domain lead to reduced levels of ubiquitination. Ubiquitination often is used to mark specific proteins for certain cellular pathways, frequently degradation (Clark et al., 2012).

BRCA1 E3 ligase on the RING domain commonly targets CTIP, estrogen receptor-alpha, progesterone receptor, as well as other proteins involved in DNA repair (Clark et al., 2012). BRCA1-mediated ubiquitination of the tumor suppressor CTIP (CTBP-Interacting Protein), rather than leading to degradation, enables CTIP to associate with damaged DNA and to contribute to G2/M checkpoint control. These functions are important to the role CTIP plays in DNA repair (Yu et al., 2006). BRCA1 ubiquitination of estrogen receptor-alpha and progesterone receptor has been implicated in degradation of both receptors, resulting in decreased signaling from each hormone (Lipkowitz et al, 2011). Because both estrogen and progesterone are linked to breast development, the effect of BRCA1 on their receptors may indicate why BRCA1 mutations are specific to breast cancer (Calvo et al., 2011). Overall, the effects of BRCA1 RING domain-mediated ubiquitination on CTIP as well as estrogen and progesterone receptors help explain why mutations in BRCA1 increase breast cancer risk (Clark et al, 2012).

Mutations in exons 11-13 of BRCA1 lead to increased cancer risk

Exons 11-13 encode over 65% of BRCA1, including nuclear localization sequences, a serine cluster domain, and binding sites for a number of proteins such as PALB2, Rad50, Rad51, and retinoblastoma protein (RB). The nuclear localization sequences on BRCA1 interact with importin-alpha machinery to transport BRCA1 from the cytoplasm to the nucleus, where DNA is located in the cell. Therefore, the nuclear localization sequences are vital for BRCA1-mediated

DNA repair, and mutations within these sequences have been linked to increased cancer risk. The serine cluster domain is phosphorylated by ATM and ATR kinases, two proteins in BASC, in response to damaged DNA. This phosphorylation signals the need for DNA repair and enables recruitment of BRCA1 to double strand breaks. Mutations within the serine cluster domain have clinically been linked to increased breast cancer incidence, possibly due to their potential to inhibit the localization of BRCA1 to damaged DNA (Clark et al., 2012).

PALB2 links BRCA1 to BRCA2, a necessary protein linkage for homologous repair of double strand breaks. Therefore, mutations in the PALB2 binding site of BRCA1 result in decreased homologous repair, which could in turn lead to pre-cancerous genomic instability. Mutations in the Rad50 binding site of BRCA1 have also been linked to increased cancer risk (Clark et al., 2012). Rad50, part of the RAD50-MRE11-NBS1 complex in BASC, participates in double strand break repair via homologous recombination (Wang et al., 2000 and Clark et al., 2012). Its interactions with BRCA1 are vital to its proper function because BRCA1 localizes RAD50 to the site of damaged DNA. Another protein involved in homologous recombination, Rad51, also binds to BRCA1 in the region of exons 11-13. Consequently, mutations in the Rad50 or the Rad51 binding domains of BRCA1 may lead to decreased DNA repair and increased cancer risk. Similarly, mutations in the BRCA1 domain that binds retinoblastoma protein have been linked to a potentially precancerous condition: decreased regulation of cell cycle progression, which can lead to genetic instability and out-of-control growth as cells bypass checkpoints needed for accurate and safe cell division. This occurs because the interaction between retinoblastoma protein and BRCA1 has been suggested to facilitate BRCA1-dependent appropriate cell cycle arrest (Clark et al., 2012).

Mutations in the BRCT domain of BRCA1 may lead to decreased DNA repair and increased cancer risk

BRCT (BRCA1 C-terminal) domain plays an important role in repair of double strand breaks, primarily by facilitating interactions between BRCA1 and other proteins phosphorylated by ATM and ATR, two aforementioned kinases that respond to DNA damage. These interactions appear to be necessary for BRCA1-mediated DNA repair and tumor suppression, as indicated by the identification of multiple cancer causing mutations within the BRCT domain (Clark et al., 2012). Additionally, the BRCT domain binds directly to DNA double strand breaks, which may contribute to the repair process (Yamane et al., 2000).

Healthy BRCA2 protein helps prevent cancer development by contributing to DNA repair

Although mutations in BRCA2 are less prevalent in breast cancer patients than BRCA1 mutations, BRCA2 mutations are also a significant risk factor for breast cancer (Ford et al., 1998). Like BRCA1, BRCA2 is involved in homologous recombination. However, much less is known about the details of BRCA2 function (Narod et al., 2004). Like BRCA1, BRCA2 also binds with Rad51, a protein that initiates homologous pairing as part of homologous repair of double strand breaks. Experiments suggest that BRCA2 regulates both transport of Rad51 into the nucleus and binding of Rad51 to DNA. BRCA2 proteins with mutations in the Rad51-binding domain are deficient in these functions, likely due to a deleterious decrease in Rad51-mediated homologous repair (Davies et al., 2001).

Mutations within DSS1-binding domain of BRCA2 are linked to breast cancer

In addition to the interaction of BRCA2 with Rad51, BRCA2 also binds with DSS1 protein. This binding appears to play an important role in the BRCA2 tumor suppressor function,

as research has linked mutations on the DSS1-binding domain of BRCA2 to increased cancer incidence (Yang et al., 2002). A later study found that the association of DSS1 with BRCA2 stabilizes the BRCA2 protein, preventing its degradation and allowing BRCA2 to continue its function of DNA repair. Therefore, mutations in BRCA2 within its DSS1 binding domain may lead to decreased genome integrity, increasing cancer risk (Li et al., 2005).

Treatment options for patients with mutations in BRCA1 and BRCA2

For those who suspect they may have mutations in BRCA1 and/or BRCA2, genetic testing is available. Following a positive test for a mutation in either of these genes, scheduling semi-annual clinical breast exams is recommended to increase the odds of early detection. An annual mammogram or MRI (magnetic resonance imaging) is also part of the recent guidelines for carriers of these mutations (Nusbaum et al., 2007). Because carriers of BRCA1 and/or BRCA2 mutations have such a high risk of developing breast cancer, certain preventative treatments are available to reduce their risk even before they develop breast cancer (Genetics, 2012).

One aggressive preventative treatment option is a bilateral prophylactic mastectomy, or the surgical removal of both breasts. This treatment has been shown to reduce a woman's risk of developing breast cancer by 90% (Rebbeck et al., 2004). Another aggressive treatment option is a prophylactic oophorectomy, or removal of both ovaries. A 2006 study suggests that the efficacy of this treatment is dependent on the type of BRCA mutation involved. Carriers of a mutation in BRCA1 who underwent a prophylactic oophorectomy experienced a 39% reduced breast cancer risk, while women with a BRCA2 mutation saw a 72% reduction in breast cancer risk as a result of the treatment (Cancer Risk Reduction, 2006). Because the ovaries are the body's primary

source of estrogen, their removal may be more likely to reduce the risk of estrogen-receptor-positive breast cancer. BRCA1 mutation carriers are more likely to develop estrogen-receptor-negative breast cancer, whereas BRCA2 mutation carriers are more likely to develop estrogen-receptor positive breast cancer. This is a possible explanation as to why women with a mutation in BRCA2 have greater risk reduction as a result of this surgery than women with BRCA1 mutations. However, preventative surgical treatments like mastectomies and oophorectomies are invasive treatment options and are therefore not always the ideal choice for everyone with BRCA1 or BRCA2 mutations (Genetics, 2012).

B. Inherited mutations in genes other than BRCA1 and BRCA2

Mutations in TP53 can increase breast cancer risk

Although mutations in BRCA1 and BRCA2 are by far the most common genetically inherited causes of breast cancer, mutations in a number of other proteins have also been linked to breast cancer (Gage et al., 2012). One such protein is p53, a protein encoded by the *TP53* gene. P53 responds to damaged DNA by causing cell cycle arrest at G1 and triggering the DNA repair process (Kastan et al., 1991). If the DNA is damaged beyond repair, p53 is also capable of causing apoptosis, or programmed cell death, of the damaged cell (Lowe et al., 1993). These functions illustrate the importance of p53 in DNA damage response. Because of the vital role p53 plays in tumor suppression, mutations within the *TP53* gene have also been implicated in increasing the risk of developing many cancers other than breast cancer, including brain cancer, leukemia, and osteosarcomas. Mutations in *TP53* can lead to development of Li-Fraumeni syndrome, a condition characterized by the development of several tumors at a young age. Those diagnosed with Li-Fraumeni syndrome have up to a 90% chance of developing breast cancer.

Although the *TP53* mutation is relatively rare in the population, it accounts for approximately 1% of inherited breast cancer cases. Genetic testing is available for *TP53*. If a mutation is detected, biannual clinical breast exams as well as annual mammograms or MRIs are recommended beginning between ages 20 and 25 due to the large increase in cancer risk (Gage et al., 2012).

Mutations in PTEN can increase breast cancer risk

Another inherited mutated gene linked to breast cancer is *PTEN*. Mutations in this gene may lead to Cowden syndrome, which is characterized by the formation of benign and malignant tumors and accounts for less than 1% of inherited breast cancer cases. Mutations in *PTEN* elevate lifetime risk for the development of breast cancer to 25-50% (Gage et al., 2012). This significant increase may be due to multiple functions of *PTEN* that contribute to overall genomic stability. One such function is the ability of *PTEN* to act as a transcriptional activator of Rad51, an aforementioned protein that works in conjunction with BRCA1 and BRCA2 to repair double strand breaks. Therefore, mutations in *PTEN* may lead to low levels of Rad51 and decreased repair of damaged DNA (Shen et al., 2007).

Additionally, *PTEN* can trigger apoptosis in response to severe DNA damage, thereby preventing a dysfunctional cell from creating mutated progeny. To accomplish this goal, *PTEN* dephosphorylates PI(3,4,5)P₃ (phosphatidylinositol-(3,4,5)-triphosphate). This prevents PI(3,4,5)P₃ from contributing to the activation of Akt, a protein kinase that induces survival signals that prevent apoptosis. Therefore, *PTEN* prevents the activation of Akt, resulting in the promotion of apoptosis (Chung et al., 2005). Genetic testing is available for *PTEN* mutations. If a mutation is found, the National Comprehensive Cancer Network recommends biannual clinical breast exams

and regular mammographies or MRIs in order to increase the odds of early cancer detection (Gage et al., 2012).

Mutations in *STK11* can increase breast cancer risk

The *STK11* gene codes for serine/threonine kinase 11 (STK11), also known as liver kinase B1 (LKB1). Like p53 and PTEN, STK11 promotes genomic stability by playing a role in apoptosis of cells with severely damaged DNA (Gage et al., 2012). STK11 activates AMPK, a protein which in turn leads to the activation of the TSC1-2 complex. The active TSC1-2 complex inhibits RHEB, a small protein that, when uninhibited, promotes mTOR signaling leading to cell proliferation and survival. When STK11 does not adequately inhibit RHEB, mTOR activity increases and cells exhibit increased potential for cancerous growth (Mahoney et al., 2008). Therefore, mutations in STK11 can increase the risk of developing different types of cancer. Individuals with mutations in STK11 have a 32% chance of developing breast cancer by age 60 (Lim et al., 2004). Genetic testing is available for STK11. Individuals with mutations in STK11 should adhere to regular testing for breast cancer, including monthly breast self-examinations, semiannual clinical breast exams, and annual mammograms (Giardiello et al., 2006).

Mutations in *CHK2* and *ATM* can increase breast cancer risk

CHK2 and ATM are proteins known to participate in the same pathways as BRCA1 and BRCA2. Consequently, mutations in CHK2, ATM, BRIP1, and PALB2 have been linked to increased breast cancer risk (Stratton et al., 2007). CHK2, also known as hCds1, co-localizes to nuclear foci with BRCA1. In response to DNA damage, CHK2 phosphorylates BRCA1 at serine 988, a specific amino acid residue on the protein. This phosphorylation is necessary for BRCA1 to both dissociate from CHK2 and to participate effectively in its DNA repair function (Lee et al., 2000). A loss of function mutation in the kinase domain of CHK2 that is responsible for this

phosphorylation of BRCA1 can lead to a twofold increase in breast cancer risk for women and a tenfold increase in risk for men (Meijers-Heijboer et al., 2002). CHK2 testing has recently become available. Women who test positively for mutations in this gene may wish to undergo more regular testing for breast cancer (Gage et al., 2012).

ATM, or Ataxia-Telangiectasia Mutated, is considered part of the BASC complex that includes BRCA1 and other proteins (Wang et al., 2000). In response to double strand breaks, ATM phosphorylates and activates p53 and BRCA1 (Shiloh, 2006). As discussed above, activation of p53 leads to p53-mediated cell cycle arrest, DNA repair, and apoptosis if needed. ATM-mediated activation of BRCA1 is necessary for localizing BRCA1 to damaged DNA, where it participates in DNA repair (Clark et al., 2012). Due to the aforementioned importance of p53 and BRCA1 in the repair of double strand breaks, ATM is vital in the appropriate response to DNA damage. Therefore, mutations in ATM can double the risk of breast cancer (Renwick et al., 2006). Individuals who test positively for mutations in ATM should undergo more aggressive screening for breast cancer (Gage et al., 2012).

Overall, genetics can play an important role in determining an individual's breast cancer risk. A woman with one first-degree relative - meaning a mother, father, sister, brother, daughter, or son - with breast cancer has double the breast cancer risk of a woman with no family history of breast cancer, while having two first-degree relatives triples a woman's risk. Inherited mutations in BRCA1, BRCA2, P53, PTEN, STK11, CHK2, and ATM are a large part of what causes this risk increase. Analyzing how these proteins prevent tumor growth helps explain why such inherited mutations may be significant risk factors for breast cancer. However, genetics do

not provide a complete picture of breast cancer susceptibility. Other factors, such as lifestyle factors, need to be considered to determine a woman's risk (What are the risk factors, 2013).

C. Oral contraceptive use

Estrogen and Progestin promote potentially precancerous cell growth

A number of studies have found a link between the use of oral contraceptives and breast cancer risk (Hunter et al., 2010). This link is likely due to the relationship between hormones in the birth control pills and breast cancer. Birth control pills typically contain estrogen and progestin (a synthetic form of progesterone), two hormones which exert many different physiological effects on breast tissue. Additionally, evidence suggests that ovarian hormones, such as estrogen and progesterone, are absolutely essential for the formation of breast cancer tumors (Nandi et al., 1995). Examining the physiological role of estrogen and progesterone at a cellular level helps reveal the mechanisms underlying the link between oral contraceptive use and breast cancer risk.

More specifically, when estrogen binds to an estrogen receptor, it triggers the Ras-Raf-MAPK (mitogen-activated kinase) pathway, which increases cell proliferation (Zivadinovic et al., 2005). Through this mechanism, estrogen can increase proliferation of epithelial cells within the breast; such rapid cell division increases the potential for accumulated mutations and resultant oncogenesis (Nandi et al., 1995). Additionally, estrogen prevents the release of cytochrome c from the mitochondria into the cytoplasm, an important step in triggering apoptosis. Therefore, increased levels of estrogen may prevent cells with damaged DNA from being destroyed, enabling their survival and potential replication. This process increases cancer risk (Morkuniene et al., 2006).

Furthermore, estrogen and progesterin in combination, as they are in birth control pills, may increase cell division more than estrogen alone (Pike et al., 1993). A 2010 study reiterated this finding by using in vivo rat models to show that estrogen and synthetic progesterone stimulate greater cell proliferation in the mammary gland than estrogen alone. Estrogen signaling in breast tissue increases expression of Areg, an epidermal growth factor receptor ligand that promotes cell proliferation within the breast. Progesterone may help stabilize the Areg protein. Additionally, progesterone works synergistically with Areg to activate cell signaling pathways that stimulate cell proliferation (Kariagina et al., 2010). Along with the cell division and cell survival effects promoted by estrogen through other aforementioned pathways, these effects increase the probability of uncontrolled growth that leads to breast cancer.

Increase in breast cancer risk is dependent upon the type of birth control used

Overall, the use of current oral contraceptives increases a woman's risk for breast cancer by 1.8%. This risk is likely due to activation of growth pathways by estrogen and progesterone in the contraceptives, which causes a marked increase in breast tissue proliferation. Ten years after discontinuing use of oral contraceptives, a woman's risk returns to normal regardless of the type of birth control used. However, different types of birth control exert different effects on breast cancer risk during contraceptive use or before this ten year period has elapsed (Hunter et al, 2010). For example, birth control pills made before 1975 had significantly higher levels of hormones than modern formulations and therefore have been implicated in increasing breast cancer risk by up to 20% (Grabrick et al., 2000). Additionally, most of the 1.8% increase in risk associated with current oral contraceptives is attributable to the pills containing levonorgestrel, a specific synthetic variant of progesterone (Hunter et al., 2010). Overall, there is

only a slight increase in breast cancer risk associated with most modern birth controls, and some studies have found no increased risk of breast cancer for oral contraceptive users (Davidson et al., 2002). Therefore, most women on modern oral contraceptives are not significantly increasing their breast cancer risk by taking birth control pills (Hunter et al., 2010). However, other types of current birth control have been linked to much greater increases in cancer risk. For example, use of the injectable birth control depo-medroxyprogesterone acetate (DMPA) more than doubles a woman's breast cancer risk (Li et al., 2012). Therefore, women concerned about their breast cancer risk may wish to take care when choosing which type of birth control to use.

D. Hormone replacement therapy

Hormone replacement therapy is associated with increased breast cancer risk

Hormone replacement therapy is the administration of hormones, usually in menopausal or postmenopausal women, to alleviate the symptoms of menopause. Typically, hormone replacement therapy involves a form of estrogen and a form of progestin in conjunction with each other (Rozenberg et al., 2013). A 2006 study found that use of hormone replacement therapy containing both estrogen and progestin increases breast cancer risk after an average of 5.6 years of use; these results are consistent with other studies that found a positive correlation between hormone replacement therapy and breast cancer risk (Anderson et al., 2006 and Rozenberg et al., 2013). The mechanisms underlying this increased risk are likely due to the proliferative effects estrogen and progesterone have on the body, more specifically increased breast tissue growth discussed above. Because of this increase in risk, women who are at high risk of breast cancer may choose not to use hormone replacement therapy.

E. Alcohol use

Alcohol use can significantly increase breast cancer risk

Alcohol consumption is strongly correlated with cancer risk. Weekly binge drinking, defined as consumption of 4-5 drinks at a time, increases breast cancer risk by 55% compared with one weekly drink. Consistently drinking large amounts of alcohol has an even more significant effect on cancer risk; drinking 22-27 drinks weekly more than doubles the risk of breast cancer compared to weekly consumption of 1-3 drinks (Morch et al., 2007). However, alcohol consumption does not need to be high to increase cancer risk. Low to moderate alcohol consumption has been linked to increased cancer risk; even drinking as little as 1 drink a day has a slight increase in breast cancer risk (Bagnardi et al., 2013). Furthermore, every additional drink consumed per day is associated with a 2% increase in breast cancer risk, although another study found the risk associated with each drink to be as high as 10% (Morch et al., 2007 and Key et al., 2006). To understand the reasoning behind this risk increase, the physiological effects of alcohol must be examined.

Alcohol use affects breast cancer risk through a number of physiological mechanisms

Alcohol exerts many effects on the body. A number of studies have linked alcohol consumption with increased estrogen levels in the body (Singletary and Gapstur, 2001). As discussed earlier, estrogen activates a number of cellular pathways that promote cell proliferation in breast tissue, potentially leading to cancer (Zivadinovic et al., 2005 and Kariagina et al., 2010). This may be the primary mechanism by which alcohol affects breast cancer risk. Additionally, alcohol consumption has been linked to increased DNA damage. One mechanism by which such damage occurs is that ethanol inhibits O-6-methylguanine-DNA

methyltransferase, a DNA repair protein; such inhibition prevents repair of DNA damage (Espina et al., 1988). Furthermore, the body metabolizes ethanol into acetaldehyde, a potent carcinogen and mutagen. Acetaldehyde can bind DNA, which can lead to DNA replication errors and therefore mutations. Acetaldehyde has also been known to inhibit proper DNA synthesis, leading to the increased incidence of mutations (Poschl and Seitz, 2004). Therefore, alcohol can exert many effects on the body that induce the kind of DNA damage that may increase cancer risk.

Adequate folate intake may counter the tumorigenic effects of alcohol intake

The most obviously effective method of avoiding the increased breast cancer risk associated with alcohol is to abstain from drinking. However, a number of studies have indicated that adequate intake of folate, vitamin B9, may negate the increased breast cancer risk associated with alcohol consumption. Folate consumption does not decrease cancer risk for non-drinkers (Zhang et al., 1999 and Baglietto et al., 2005). Folate is needed for accurate DNA replication and proper DNA methylation (Kamen, 1997 and Baglietto et al., 2005). Acetaldehyde may decrease folate levels in the body; such a decrease may deleteriously affect DNA replication and methylation. Having adequate dietary intake of folate may help to restore the functions folate normally performs in the body, thereby countering some of the negative effects of alcohol. However, other unknown mechanisms may be at play regarding the mitigation of alcohol-related breast cancer risk by folate intake. Although the complete reasoning for such mitigation is currently uncertain, the positive effects of folate should not be ignored, as folate intake may be a viable option to reduce risk of breast cancer for drinkers (Baglietto et al., 2005).

F. Excess Weight and Obesity

Excess Weight and Obesity have been linked to increased breast cancer risk

In the United States, about 69.2% of adults are overweight, while approximately 35% of the population is obese (Obesity and Overweight, 2012). Therefore, the association between obesity and breast cancer is one that is unfortunately relevant for many Americans. Overweight postmenopausal women have a 12% breast cancer risk increase compared to postmenopausal women of a healthy weight (Eliassen et al., 2006). Obesity increases a woman's chance of developing triple-negative breast cancer (breast cancer that does not express an estrogen receptor, a progesterone receptor, or a Her2/neu receptor) by 35% and of developing estrogen-receptor positive breast cancer by 39% (Phipps et al., 2011). Furthermore, a number of studies have linked weight gain during adult life to postmenopausal breast cancer risk; a woman's risk for developing postmenopausal breast cancer approximately doubles following a weight gain of 44 pounds or more after the age of 18 (Huang et al., 1997). Another study suggests that every additional point increase beyond a healthy BMI elevates breast cancer risk by 2% (Bergstrom et al., 2001). To fully elucidate the connection between breast cancer risk and excess weight, the physiological effects of excess body weight must be examined. Such scrutiny reveals that obesity affects multiple pathways associated with cancer.

Inflammatory adipokines may increase cancer risk

Adipokines are cytokines secreted by adipocytes, the main cell type that composes adipose tissue. Obese individuals, presumably due to larger amounts of adipose tissue, typically exhibit higher levels of local and circulating inflammatory adipokines. These adipokines induce recruitment of T cells and macrophages, the latter of which stimulates the release of more cytokines. In this way, adipose tissue in obese individuals promotes high concentrations of cytokines. Of the more than 50 adipokines produced by adipose tissue, several may be linked to

cancer risk (Gilbert and Slingerland, 2012). One cytokine present in adipose tissue is IL-6, or interleukin 6. IL-6 can activate a Janus kinase (JAK) pathway, which in turn induces Ras-MAPK and PI3K (phosphatidylinositol 3-kinase) signaling. The tumorigenic effects of IL-6 include promoting proliferation of epithelial tissue and countering apoptotic signals, which could lead to the uncontrolled growth that characterizes cancer (Bromberg and Wang, 2009).

Leptin is another adipokine that is found in higher levels in obese individuals and contributes to the development of cancer through a myriad of effects on cellular metabolism (Ray and Cleary, 2012). For example, leptin promotes expression of estrogen receptor- α , which enables increased estrogen signaling (Yu et al., 2010). As discussed above, estrogen stimulates proliferation of breast tissue, thereby increasing breast cancer risk (Zivadinovic et al., 2005 and Kariagina et al., 2010). Additionally, leptin stimulates cell division through signaling involving the ERK protein; leptin also reduces apoptosis via enhanced Akt signaling. Both increased cell division and decreased apoptosis increase the risk of tumorigenesis. Leptin also downregulates expression of p53, an aforementioned DNA repair protein that is central to proper maintenance of genetic material. Lower levels of p53 decrease the stability of the genome, increasing the risk of cancer (Ray and Cleary, 2012). Overall, leptin is capable of exerting a number of tumorigenic effects that contribute to the association between obesity and increased breast cancer risk.

Insulin resistance associated with obesity may contribute to breast cancer risk

Obesity is a well-established causative agent of insulin resistance, a condition in which insulin pathways do not properly function. The body compensates by overproducing insulin. Higher levels of circulating insulin stimulate PI3K and MAPK signaling pathways, both of which promote cell proliferation (Ray and Cleary, 2012). Such signaling increases the

probability of uncontrolled cell division that destabilizes the genome and is characteristic of tumor formation (Zivadinovic et al., 2005). Additionally, insulin resistance leads to increased production of insulin-like growth factor-I (IGF-I), which increases cell proliferation via PI3K and MAPK signaling pathways in the same manner as insulin. Therefore, obesity-induced insulin resistance plays a role in obesity's positive correlation with breast cancer (Ray and Cleary, 2012).

Obesity is correlated with higher estrogen levels that may promote tumorigenesis

Higher aromatase expression can be induced by a number of the inflammatory cytokines and adipokines that are present at higher levels in obese women. Aromatase is an enzyme required for estrogen synthesis; greater amounts of aromatase will consequently lead to greater levels of estrogen in the body (Gilbert and Slingerland, 2012). Therefore, it may be unsurprising that estrogen levels are higher in obese postmenopausal women than in postmenopausal women of a healthy weight (Key et al., 2003). As discussed above, estrogen induces cell proliferation within breast tissue, which increases breast cancer risk (Zivadinovic et al., 2005 and Kariagina et al., 2010). As a result, higher estrogen levels are also a factor when determining how obesity affects breast cancer risk.

Attainment of a healthy weight negates the risk associated with obesity

Weight loss is the primary solution to avoid the cancer risk that accompanies obesity. Women at healthier weights have lower levels of adipokines, lower risk of insulin resistance, and lower estrogen levels than obese women, thereby allowing women of a healthy weight to be at lower risk for breast cancer (Gilbert and Slingerland, 2012; and Ray and Cleary, 2012). The process of losing weight may be linked to reduced cancer risk even when the amount lost is

relatively small; a study found that losing 4-11 pounds following menopause may decrease breast cancer risk by up to 20% (Eliassen et al., 2006). Therefore, obese women seeking to reduce their breast cancer risk should take steps to attain a healthy weight. Exercise, as an aid to weight loss, may help reduce cancer risk. Other studies have found that exercise carries with it additional risk-reducing benefits even when no weight is lost; such benefits include lowering estrogen levels (Friedenreich et al., 2010). Regardless of whether or not one is obese, regular exercise may cause a 10-20% decrease in breast cancer risk (Tehard et al., 2006).

G. Diet

i. Foods that may increase breast cancer risk

Fat intake

The majority of studies linking certain dietary factors to breast cancer are controversial; many dietary studies are characterized by a number of confounding factors including small sample size and diets that are potentially too homogenous to generate noticeable effects on cancer risk. Despite the controversy, a number of reputable studies suggest an association between dietary factors and cancer. Fat intake is an example of such a dietary factor. While some studies have found no link between increased fat intake and breast cancer risk, others have implicated it as a potential risk factor (Wynder et al., 1997). Since the 1950's, animal studies have suggested that a high-fat diet stimulates the development of tumors within the mammary tissue of mice (Tannenbaum and Silverstone, 1953). These findings are supported by international studies, which found a correlation between higher per capita intake of fat and higher rates of breast cancer. Additionally, analysis of 12 case-control studies found a 50% increase in breast cancer risk associated with a high fat diet in postmenopausal women (Hankin,

1993). Another study calculated that postmenopausal women who decrease their dietary fat intake to 50% of the average United States intake may experience a 2.5-fold reduction in breast cancer risk (Prentice and Sheppard, 1990).

Several physiological mechanisms could account for these findings. One suggestion is that higher dietary fat intake corresponds with greater estrogen production (Prentice et al., 1990). In addition to producing more estrogen, high dietary fat leads to higher serum free fatty acid levels, which can promote displacement of estrogen from serum albumin. This process can increase levels of bioavailable estrogen, meaning that this estrogen is free to activate estrogen receptors (Bruning and Bonfrer, 1986). Increases in both estrogen production and free estrogen can upregulate the effects of estrogen on the body. As discussed above, estrogen promotes cell proliferation within breast tissue that can increase the probability of developing cancer (Zivadinovic et al., 2005 and Kariagina et al., 2010). Additionally, certain fatty acids have been shown to affect gene expression that may affect cancer risk. For example, linoleic acid can decrease p53 expression in rat cells. Such suppression of p53, a major tumor suppressor and DNA repair protein, may account for some of the cancer risk associated with a high fat diet (Ronai et al., 1995).

ii. Foods that may decrease breast cancer risk

Phytoestrogens

Phytoestrogen is a compound naturally found in certain plants that bears a structural resemblance to estrogen. Soybeans or foods containing soy are the most significant dietary source of phytoestrogens (Kurser and Xu, 1997). Although some studies have found no link between phytoestrogen intake and breast cancer risk, other studies have found a relationship

(Adlercreutz, 2002). For example, one study found Chinese women who consume soy foods less than once a week have double the risk of breast cancer relative to women who eat soy daily (Lee et al., 1991). A more recent study indicated that high soy intake during adolescence could decrease breast cancer risk later in life (Shu et al., 2001).

Partially because the relationship between phytoestrogens and breast cancer is unconfirmed, the physiological mechanisms through which phytoestrogen may decrease breast cancer risk remain hypothetical. Despite this, a number of possible mechanisms have been proposed. One theory is that phytoestrogens preferentially bind to estrogen receptor beta ($ER\beta$) as compared to estrogen receptor alpha ($ER\alpha$); this finding has been confirmed by in vitro testing that reveals phytoestrogens have ten times the affinity for $ER\beta$ as opposed to $ER\alpha$ (Kuiper et al, 1998). $ER\beta$ may counter the effects of $ER\alpha$, including the proliferative effects $ER\alpha$ usually exerts (Weihua et al., 2000). Regardless of the mechanism by which soy consumption may lower breast cancer risk, the overall link between phytoestrogens and breast cancer risk remains contentious (Adlercreutz, 2002).

Brassica vegetables

Brassica vegetables, also known as cruciferous vegetables, include broccoli, cabbage, kale, cauliflower, and brussels sprouts. Women who eat approximately 1.5 servings of these vegetables a day experience a 42% decrease in breast cancer risk compared to women who do not consume brassica vegetables. When looking for an explanation for this decreased risk, the physiological effects of brassica vegetable consumption on estrogen metabolism must be examined. 16α -hydroxyestrone (16HE), a product of estrogen metabolism, promotes cell proliferation through binding an estrogen receptor (Fowke et al., 2000). Additionally, 16HE may

directly lead to DNA damage; both of these effects can increase the risk of breast cancer (Telang et al., 1992 and Fowke et al., 2000). An alternative estrogen metabolite, 2-hydroxyestrone (2HE) has a much weaker affinity for estrogen receptors and does not exert the potentially cancer-inducing effects of 16HE. The pathways to produce 16HE and 2HE are irreversible, and therefore a low ratio of 2HE:16HE (2:16) in the urine corresponds with higher breast cancer risk, while a high 2:16 ratio corresponds with lower risk. Increased brassica vegetable consumption was linked with increasing the 2:16 ratio. This effect could account for why consumption of brassica vegetables may decrease breast cancer risk (Fowke et al., 2000).

Conclusion

Overall, several risk factors for breast cancer have been established. This review provides a basic overview of the genetic component of breast cancer risk. Other factors that may be preventable, including use of oral contraceptives, hormone replacement therapy, and alcohol were also discussed. Obesity and diet are two other factors described in this review that can be controlled to reduce risk. The discussion includes physiological mechanisms through which such factors increase breast cancer risk, the risk associated with each factor, and the possible ways each risk factor may be addressed or prevented. Because only about 30% of breast cancers are attributable to established breast cancer risk factors like those discussed here, further research is needed to identify more risk factors. Another factor influencing this statistic is that a woman's individual risk for breast cancer is difficult to assess, as she may have multiple risk factors, or risk factors of which she is not aware (Madigan et al., 1995). Additionally, some of the risk factors in this review are still under examination, such as consumption of phytoestrogens

contained in soy; further investigation will help to determine how such factors may affect breast cancer risk (Adlercreutz, 2002).

In conclusion, it is important to keep in mind that no single risk factor or combination thereof is a guarantee of breast cancer development. However, knowledge of the potential risk factors can still be helpful to high risk women, who may wish to be more carefully monitored for the development of breast tumors. Additionally, such knowledge may allow women to take potentially preventative measures against breast cancer. Finally, continued research regarding these risk factors is of vital importance, as it could enable early detection or even prevention of breast cancer.

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