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A COMPREHENSIVE LITERATURE ANALYSIS OF CAUSES AND TREATMENT OPTIONS FOR HEREDITARY BREAST CANCER

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Abstract:

Mutations in the BReast CAancer suspectibility genes BRCA 1 and 2 account for approximately 10% of breast cancers and 15% of ovarian cancers. Individuals with a mutation in these genes (and a resulting impairment in the function of these genes in e.g. DNA repair and estrogen signaling) have a much higher risk for getting these cancers than the population at large. This review aims to provide comprehensive information for BRCA mutation carriers on currently available options to lower the risk of getting cancer. The review summarizes interaction of lifestyle/environmental factors, such as diet, alcohol consumption, and hormonal supplements, in key pathways and mechanisms involved in cancer onset via a role in estrogen metabolism and DNA repair. This review furthermore evaluates promising future research directions and concludes that (i) more BRCA testing needs to be conducted on broader populations, (ii) BRCA1 and BRCA2 need to be analyzed separately as they exhibit distinct characteristics, and (iii) studies on lifestyle factors need to include multiple, synergistically acting factors and lifestyle history from many years prior to diagnosis. Additionally, (iv) molecular diagnostics and gene therapy provide promising results for hereditary cancers and need to be moved forward as quickly as possible. Furthermore, (v) research seems to be hindered by a lack of interdisciplinary collaboration, and should greatly benefit from a more synergistic effort. Finally (vi) lifestyle and diet changes can be made immediately to lower cancer risk for BRCA mutation carriers.

1. Introduction

Breast cancer is one of the most common cancers and affects millions of people worldwide (Siegel et al., 2012). Breast cancer awareness in general, as well as funding for research and life-saving early detection, has been on the rise (Siegel et al., 2012). However, little is known about hereditary breast cancer associated with BRCA (BReast CAncer susceptibility genes). BRCA1 and 2 are "tumor suppressor genes" that, when rendered nonfunctional by mutations, cause high penetrance (percent of people with the gene who actually get cancer) of breast and ovarian cancer (Christopoulou and Spiliotis, 2006). For my thesis, I conducted a comprehensive literature review of BRCA to identify what is known today and on what areas future research should focus. I reviewed and summarized current knowledge of the roles of (1) genetics (population and molecular genetics) and (2) environment (diet and hormonal supplements), as well as where efforts to utilize (3) molecular diagnostics and gene therapy stand at this time. I concluded that there is evidence for roles of multiple different genetic and environmental factors in BRCA-associated cancer (and breast cancer overall), but that research has been focused on each factor in isolation and now urgently needs to be conducted in a comprehensive, integrative way to assess synergistic effects among different factors. Furthermore, many current studies treat BRCA1 and BRCA2 as equivalent, despite the fact that these two genes generate tumors with different characteristics (Foulkes et al., 2004) and should therefore be analyzed individually. I furthermore identify promising targets of future research on molecular diagnostics and gene therapy. Finally, I identify lifestyle changes that BRCA mutation carriers can make immediately to lower risk for cancer.

2. Defining BRCA

As stated above, BRCA1 and 2 stand for BReast CAncer susceptibility genes 1 and 2, and are tumor suppressor genes everyone possesses in his/her own genome (Christopoulou and Spiliotis, 2006). Tumor suppressor genes are genes that, in their normal, un-mutated form, help to suppress tumor formation and prevent cancer via key pathways. Hundreds of mutations in these two genes have been identified (Borg et al., 2010). Such mutations in these two genes are significantly correlated with a high risk for breast and ovarian cancer, and also exhibit apparent correlations with increased risks for cervical, uterine, pancreatic, stomach, peritoneal, and prostate and testicular cancer in men (Brose et al., 2002).

While DNA naturally accumulates mutations over time (Hanahan and Weinberg, 2000), mutations are normally either removed via repair of mutated DNA, or mutated cells are not allowed to divide (through cell cycle checkpoints controlling cell division rate) and/or are committed to programmed cell death (apoptosis) (Hanahan and Weinberg, 2000). It is when cells sustain mutations specifically inhibiting the latter key protective pathways (e.g. DNA repair or control of cell proliferation and cell death) that cells with mutated DNA are able to divide uncontrollably and produce cancer (Hanahan and Weinberg, 2000). Functional copies of BRCA1 and BRCA2 apparently prevent cancer via e.g. DNA repair (Boulton, 2006) and control of estrogen signaling (Li, 2007). Specifically, DNA repair proceeds via DNA double strand break repair by homologous recombination and by locating double strand breaks, nucleotide excision repair, cell cycle checkpoint control, ubiquitylation of proteins, chromatin remodeling, and more, with all of the latter processes being involved in tumor suppression (Boulton, 2006). Control of

estrogen signaling by functional BRCA genes involves e.g. an inhibitory effect on estrogen signaling via estrogen receptor-alpha (Li, 2007; for more detail, see below). A faulty, mutated copy of one of the BRCA genes means that the body has fewer tools to prevent cancer formation, and the risk of breast and ovarian cancer thus increases, presumably especially if BRCA mutations are combined with other factors (or mutations) that increase cell division rate, prevent programmed cell death, and/or prevent DNA repair.

The two BRCA genes are inherited in an autosomal dominant manner, meaning that they are located on chromosomes (two different autosomal chromosomes) other than sex chromosomes, and that just one faulty copy (as opposed to two copies in a recessive disorder) of the dominant gene is enough to increase cancer risk (Christopoulou and Spiliotis, 2006). Thus, only one mutated copy of the gene needs to be inherited (from one parent) to result in increased susceptibility for breast and ovarian cancer.

The BRCA genes exhibit a high, but not complete, level of penetrance (Christopoulou and Spiliotis, 2006), as the percentage of individuals with a faulty copy of the gene that get cancer. In the normal population, breast cancer risk is around 12% and ovarian cancer risk is around 1.4% (Siegel et al., 2012). For individuals with a BRCA mutation, around 60% will develop breast cancer and around 15-40% will develop ovarian cancer before age 75 (Christopoulou and Spiliotis, 2006).

Although the BRCA genes have similar names and related function, they do exhibit distinct characteristics in the majority of the tumors they produce (Folks et al., 2004). One such difference is that around 80% of BRCA 2 tumors contain estrogen receptors (are estrogen receptor positive, ER+), while only 20% of BRCA1 tumors are

ER+ (and 80% are ER-, and contain little to no estrogen receptors) (Folks et al., 2004). The presence of estrogen receptors is important for impacts on tumor survival since estrogen stimulates breast tumor growth (Anderson, 2002). Estrogen-receptor-positive (ER+) tumors respond to the presence of estrogen with cell division and increased growth (Anderson, 2002). Estrogen-receptor-negative (ER-) tumor cells exhibit few or no estrogen receptors, and estrogen is thus unable to stimulate cell division and tumor growth (Anderson, 2002). This distinction between BRCA1 and 2 is important for the following discussion of the influence of lifestyle on a BRCA mutation carrier's risk. Furthermore, normal BRCA1 suppresses estrogen signaling, and thereby inhibits the effect of estrogen in stimulating DNA synthesis and cell proliferation (Li, 2007; Santen, 2003, 2009; Welboren, 2009). Therefore, individuals with a faulty BRCA are highly sensitive to estrogen.

3. Geographic Distribution of BRCA Genes in Human Populations

Inherited copies of mutated BRCA genes have been found in human populations worldwide and are not confined to a single population, race or ethnicity, as established by BRCA studies conducted in e.g. Vietnam (Ginsburg, 2011), Russia (Suspitsin et al., 2009), the Philippines, Southern China, Japan, Eastern India (Liede and Narod, 2002), Brazil (Machado and Almeida, 2011), Morocco (Laraqui et al., 2013), Costa Rica (Gutierrez Espeleta et al., 2012), Canada (Dennis, 2011), the United States (Gallion and Smith, 1994), France (Caputo et al., 2012), Mexico (Calderon-Garciduenas et al., 2005), Puerto Rico (Dutil et al., 2012), Czech Republic (Zikan, 2005), Venezuela (Lara et al., 2012), Greece (Koumpis et al., 2011) and more. However, certain populations were

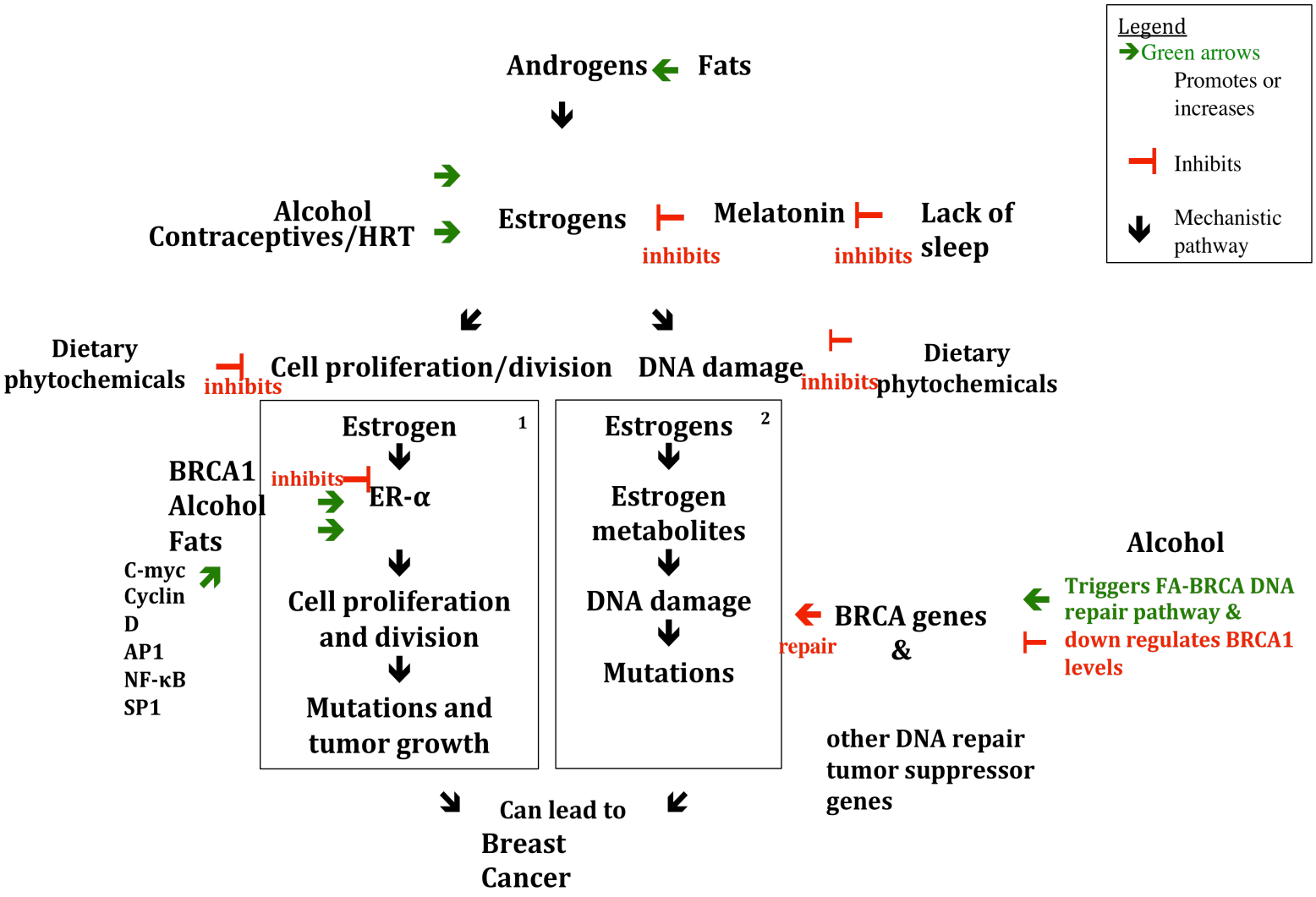
reported to have a higher prevalence of BRCA mutations, with the best known example being the Ashkenazi Jewish population, in which 1 in 40 individuals tests positive for a BRCA mutation (Struewing et al., 1997; Warner et al., 1999). However, the Netherlands, Sweden, Iceland and a population of French Canadians all exhibit high prevalence levels as well (Tonin, 2006; Ferla, 2007; Tryggvadottir, 2006).

The "founder effect", loss of genetic variation upon establishment of a new population by a few founding individuals, may be responsible for these high prevalence levels (Ferla et al., 2007). The founder effect not only creates an initial loss of genetic diversity from the original population, but also continues to create inbreeding and accumulation of deleterious mutations. The founder effect has been implicated in high BRCA prevalence of certain populations since many individuals share only a few common BRCA mutations in these populations (Ferla et al., 2007; Gutierrez Espeleta et al., 2012; Laraqui et al., 2013).

Although it is known that some populations have higher prevalence levels for BRCA than others, the exact BRCA prevalence level is unknown for many countries and ethnicities (although, see population references above). Furthermore, little is known about causes for differences in prevalence level other than the founder effect. External factors related to the physical environmental, such as diet, hormone supplement use, and pollutants, are likely to contribute to such differences via their effect on components of key pathways controlling cell division, programmed cell death, DNA repair, DNA methylation (involved in epigenetic effects that can modify the genome for a few generations), and estrogen metabolism and signaling (Hanahan and Weinberg, 2000; Surh, 2003; Gullett et al., 2010; for more detail, see section 4 below). In addition, unique

genetic interactions may also contribute to this difference in populations. Genes other than BRCA, such as those encoding growth factors and receptors, have been shown to have a multiplicative effect on breast cancer risk (Henderson and Feigelson, 2000; Henningson, 2011). I speculate that certain human populations may have higher or lower rates of these additional genes contributing to different risk levels for this polygenic disease. Few studies exist on this subject to date, and the few that do typically involve testing families of related individuals, and thus do not have a large enough sample size and statistical power to allow generalizations (Liede and Narod, 2002; Gutierrez Espeleta et al., 2012; Laraqui et al., 2013). The solution to this problem/limitation is testing of a large numbers of individuals, including random testing of the population at large rather than testing of only high-risk BRCA carriers with either a family history of the disease, or a diagnosis with breast or ovarian cancer at a young age. I conclude that such broad-scale studies of prevalence rates combined with penetrance and survival rates are urgently needed.

Figure 1


Legend
Green arrows
Promotes or increases
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Mechanistic pathway
Androgens
Fats
Alcohol
Contraceptives/HRT
Estrogens
Melatonin
Lack of sleep
inhibits
inhibits
Dietary phytochemicals
inhibits
Cell proliferation/division
DNA damage
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Dietary phytochemicals
1
Estrogen
BRCA1
Alcohol
Fats
inhibits
ER-α
C-myc
Cyclin D
AP1
NF-κB
SP1
Cell proliferation and division
Mutations and tumor growth
2
Estrogens
Estrogen metabolites
DNA damage
Mutations
repair
BRCA genes &
other DNA repair tumor suppressor genes
Alcohol
Triggers FA-BRCA DNA repair pathway &
down regulates BRCA1 levels
Can lead to
Breast Cancer

Figure 1: Schematic depiction of key pathways involved in breast cancer formation, their relationship to BCRA mutations, and their modulation by environmental factors. The two boxes shown focus on estrogen signaling leading to cell division (left box 1) and the involvement of estrogen metabolites in causing DNA damage (right box 2). Black arrows indicate the steps of the mechanistic pathways; green arrows indicate stimulation; red arrows and red T indicate inhibition. The BRCA on this scheme refer to normal un-mutated BRCA genes. ER-aplha= estrogen receptor alpha, HRT= Hormone replacement therapy: used to reduce menopausal symptoms, SP1: "specificity protein 1", AP1: "activating protein 1" AP1, NF-kB: "nuclear factor" all supporting cell proliferation, angiogenesis (development of new blood vessels supporting tumor growth), and survival and differentiation of cells. c-myc and Cyclin D= both known as cancer-promoting genes when mutated, promote cell division. The FA-BRCA pathway is a DNA damage-activated signaling pathway that controls DNA repair and cell cycle checkpoints. (Anderson, 2002; Killinger, 2005; Li, 2007; Gorski, 2009;Welboren, 2009; Santen, 2009; Dennis, 2011; Cibula, 2011; Brooks and Zakhari, 2013)

4. Comprehensive overview of lifestyle differences and their implications for BRCA mutation carriers

Figure 1 shows estrogen as a key component in cancer formation acting via stimulation of either cell proliferation or DNA damage (Santen, 2009). Estrogen can bind to estrogen receptors (e.g. ER-alpha) and promote DNA synthesis, cell proliferation, and cell division (Box 1); estrogen metabolites can promote DNA damage that, in the absence of sufficient DNA repair, can lead to the onset of cancer as a result of mutations in key tumor suppressor genes (Box 2) (Anderson, 2002; Santen, 2009).

Figure 1 also depicts functions of BRCA genes; normal BRCA1 inhibits ER-alpha signaling (Li, 2007; Gorski et al., 2009). The relationship between BRCA1 and estrogen signaling is complex; estrogen increases the expression of normal BRCA1 and normal BRCA1 simultaneously ***induces*** ER-alpha synthesis and ***inhibits*** subsequent ER-alpha signaling (Gorski et al., 2009).

Figure 1 also shows how various lifestyle factors addressed in this review affect key pathways leading to breast cancer. Alcohol consumption, certain dietary fats such as omega-6 polyunsaturated fats (which promote cell division), and saturated fats (which promote obesity and production of estrogen in these fat cells), and hormonal supplements all increase the levels of estrogen and/or cell division (Killinger, 2005; Dennis, 2011; Cibula, 2011). Alcohol is thought to increase circulating estrogen levels and may also stimulate signaling pathways leading to cell division (Brooks and Zakhari, 2013). Furthermore, the endogenous hormone melatonin inhibits estrogen production, and lack of sleep is known to cause disruption in melatonin production (Mihu, 2009; Merklinger-Gruchala, 2008). In addition, a multitude of dietary compounds ("phyto"-chemicals for "plant"-chemicals found in fruits, vegetables, herbs, and spices) inhibit multiple components of (i) signaling pathways controlling cell division as well as (ii) estrogen biosynthesis/signaling, (iii) angiogenesis (formation of cancer cell-supporting blood vessels), and (iv) stimulate programmed cell death and DNA repair (Surh, 2003; Kotsopoulos and Narod, 2005; Gullett et al., 2010; Vera-Ramirez et al., 2013)

In addition, multiple genetic interactions can affect estrogen levels and production or suppression of estrogen receptors (Fig. 1). A review by Welboren (2009) summarizes interactions between estrogen and estrogen-receptor-alpha (ER-alpha) and activation of transcription factors (e.g. "specificity protein 1", "activating protein 1" AP1, and "nuclear factor" NF-kB) supporting cell proliferation, angiogenesis (development of new blood vessels supporting tumor growth), and survival and differentiation of cells. Additional ER-alpha-regulated genes promoting cell division include c-myc and Cyclin D (both

known as cancer-promoting genes when mutated; Dubik et al., 1987; Altucci et al., 1996).

4. 1. Dietary Factors Associated with Overall and BRCA-related Cancer Risk

Many studies have been conducted on the effects of diet on overall breast cancer risk in the normal population. A diet low in fat and alcohol and high in fiber, vegetables and soy is thought to lower overall breast cancer risk (Morch, 2007; Ellison, 2001; Linos et al., 2010; Patterson et al., 2010; Ferrari et al., 2013). These latter findings are consistent with the multiple roles of phytochemicals and other dietary factors in modulating cancer-related key pathways (Fig. 1).

The present study focused on the effect of diet on BRCA mutation carriers, to evaluate whether similar or different conclusions may apply to the latter population compared to the normal population. A study done in Canada addressed whether diet quality affected breast cancer risk for BRCA mutation carriers (Ghadirian, 2009). Many French-Canadians, including 738 with diagnosed breast cancer and involving 38 BRCA carriers, were included in the latter study. Assessment of diet through a food questionnaire revealed that a diet rich in vegetable and fruit diversity was correlated with a reduced risk for breast cancer for BRCA mutation carriers (Ghadirian, 2009). Earlier studies by the same author showed that "healthy eating" in general, as described by the Canadian Healthy Eating Index and the Diet Quality Index-Revised, is also correlated with a reduced breast cancer risk for BRCA mutation carriers (Nkondjock and Ghadirian, 2007). These "healthy" diets involve consumption of diverse fruits, vegetables, grains as well as dairy, poultry and fish. An even earlier study had reported that a lower Body Mass Index (ratio of weight to height) and/or the loss of weight between the ages of 18

and 30, was correlated with a reduced breast cancer risk in BRCA mutation carriers (Kotsopoulos, 2005). This observation is consistent with findings by Lagiou (2009) in Greece that women who ate more mono- and polyunsaturated fats (as opposed to saturated fats) in the year prior to their diagnosis had tumors that expressed higher levels of estrogen-receptor-alpha. This latter study, along with another study on polyunsaturated fats in 2005 (Hilakivi-Clarke, 2005) suggests that high levels of specifically omega-6 fatty acids increase cell proliferation and therefore risk for breast cancer.

Furthermore, an inverse relationship between consumption of high levels of fruits and vegetables with a lower risk for cancer is supported by hundreds of studies (Surh, 2003). Recent research is focused on phytochemicals found in vegetables and their regulation of the pathways in Boxes 1 and 2 of Figure 1 (Anderson, 2002; Surh, 2003; Gullett et al., 2010). Such dietary phytochemicals can help prevent cancer by promoting numerous tumor suppressor genes and repressing oncogenes, inhibition of angiogenesis (the production of new blood vessels by cancerous tumors which promotes rapid growth), promotion of DNA repair, up regulation of immune responses and more (Gullett et al., 2010).

The effect of alcohol consumption on BRCA mutation carriers is another lifestyle factor that has been implicated (see Fig. 1; Dennis, 2011). Only a few studies have addressed how alcohol affects BRCA mutation carriers. A study by Dennis (2011) in Canada addressed the effects of alcohol on BRCA mutation carriers separately for the two genes involved, i.e. BRCA1 and BRCA2, and reported no significant relationship between alcohol consumption and breast cancer in carriers of BRCA1 mutations (Dennis, 2011). On the other hand, drinking red wine was suggested to be beneficial for these

latter individuals, perhaps due to a beneficial effect of the phytochemical resveratrol found in red wine (Fustier et al., 2004; Delmas et al., 2006).

Dennis (2011) found that overall increased alcohol consumption, specifically ***excluding*** red wine (with its high resveratrol content), was correlated with a ***higher*** breast cancer risk for carriers of BRCA2 mutations. The latter study suggests that the effects of alcohol on breast cancer risk are greater specifically for carriers of BRCA 2 mutations than for BRCA1 mutation carriers or the general population without BRCA mutations. Furthermore, this increased risk for BRCA2 mutation carriers was greater in women with a Body Mass Index over 25 and in women who've had a child (Dennis, 2011). A relationship between obesity and breast cancer may involve fat cells that can contribute to estrogen levels by converting androstenedione to estrone in adipose tissue (Killinger, 2005).

The conclusion that alcohol differentially affects certain BRCA mutation carriers is further supported by a study showing that alcohol damages DNA and specifically activates the FA-BRCA pathway for DNA repair (Abraham, 2011; see Fig. 1). The FA-BRCA pathway is a DNA damage-activated signaling pathway that controls DNA repair and cell cycle checkpoints. Furthermore, alcohol down-regulates mRNA and protein levels of BRCA1 (Fan, 2000). Additionally, alcohol increases (i) circulating estrogen levels in the body (Reichman et al., 1993; Dorgan et al., 2001; Fan, 2000) and (ii) the sensitivity of estrogen receptors to estrogen levels (Fan, 2000). BRCA1 normally suppresses estrogen-receptor-alpha signaling, and alcohol partially reverses the repression.

As stated above, BRCA 1 and BRCA 2 differ in their estrogen-receptor status, with around 80% of BRCA 2 tumors being ER+ (showing a high presence of estrogen receptors) while 80% of BRCA1 tumors are ER-. In comparison, around 60% of tumors found in individuals not carrying a mutated BRCA gene are ER+ (Singletary, 2001). These features offer an explanation of the results of Dennis (2011), where (the ER-positive) BRCA2 mutation carriers, but not (the ER-negative) BRCA1 mutation carriers, were found to have an increased risk for cancer from alcohol consumption. Li (2009) further confirms the correlation between a higher risk of ER+ breast cancer and alcohol consumption. However, the alcohol history of patients recorded by Dennis (2011) was only taken during the year prior to breast cancer diagnosis and it is known that cancer grows in the body for long periods of time before diagnosis (Patrone, 2011). This is a major design limitation in the study by Dennis (2011) and other lifestyle questionnaire studies that only obtain recent history. I conclude that future studies are needed that compare life-long alcohol consumption to the risk for breast cancer.

4. 2. Hormonal Supplements and BRCA

Although it seems intuitive that hormone supplements would affect the occurrence of breast and ovarian cancer, as cancers of the reproductive organs, limited research is available on the specific effects of hormones on BRCA mutation carriers. Nevertheless, this limited research proves insightful for ovarian cancer risk.

There is evidence that estrogen-based contraception can protect against ovarian cancer, while possibly stimulating the risk for breast cancer (see below). Many studies have suggested a protective effect of oral contraceptives for ovarian cancer risk in the

normal population, and six studies specifically addressed the effect of oral contraceptives on BRCA mutation carriers. Five out of these six studies concluded that ovarian cancer risk is reduced as a result of oral contraceptive use for both BRCA 1 and BRCA 2 mutation carriers (Narod et al., 2002; McGuire et al., 2004; Whittemore et al., 2004; McLaughlin et al., 2007; Antoniou et al., 2009). This protective effect seems to increase with duration of contraceptive use. The mechanism of this protective effect has not been fully elucidated, but it has been suggested that inhibition of ovulation, suppression of gonadotropin levels, and increase of progestin levels may all play a role in protecting against ovarian cancer (Cibula, 2011). The "incessant ovulation hypothesis" assumes ovulation itself to increase ovarian cancer risk because of "damage" to the epithelium (Fathalla, 1971; Purdie, 2003; Murdoch, 2005). Another mechanistic hypothesis proposes that ovarian mutagenesis is caused by excessive gonadotropin levels that naturally fluctuate with a women's menstrual cycle (Cibula, 2011). On the other hand, progesterone, one of the hormones taken in oral contraceptives, has been linked to up-regulation of the tumor suppressor p53, apoptosis, and lowered cell proliferation, all functions aiding in tumor suppression (Bu et al., 1997; Cibula, 2011).

In contrast to the situation for ovarian cancer, oral contraceptives may increase the risk for breast cancer; Iatrakis (2011) provides evidence for such an effect in BRCA1 and 2 mutation carriers. Cibula (2011) showed an increase of breast cancer only for BRCA1 mutation carriers. This latter effect may be due to increased levels of estrogen affecting tumor growth in ER+ breast tumors (Anderson, 2002). However, the literature on this point is not consistent; while most studies suggest an increased risk for longer duration of oral contraceptive use, others claim no significant increase in risk (Iodice,

2010; Narod et al., 2002). It thus appears that more studies are needed that separate effects on carriers of the two BRCA genes.

Furthermore, many women are interested in how hormone replacement therapy, i.e. taking estrogen and/or progestin to relieve menopausal symptoms, will affect their cancer risk. This is particularly interesting to BRCA mutation carriers, as many receive recommendations from their doctors to undergo prophylactic surgery, i.e. removal of target organs as a prevention tool, which can trigger early menopause. Women who have their ovaries and uterus removed as a preventive measure due to their BRCA-positive status need to know if hormone replacement therapy will increase their cancer risk. A few studies on how hormone replacement therapy affects BRCA mutation carriers have been conducted and conclude that there is no increase in risk (Kotsopoulos et al., 2006; Biglia, 2008). However, these studies typically did not separate BRCA1 and BRCA2 mutation carriers in their analysis. Again, for conclusive results these two groups must be separated due to their differences in tumor characteristics with respect to hormone receptors.

4.3 Sleep Deprivation/Shift Work and Increased Breast and Ovarian Cancer

A correlation has been shown between working night shifts and breast cancer risk (Schernhammer et al., 2001; Megdal et al., 2005). This increased risk has been suggested to be caused by a disruption in natural circadian rhythm (Mahoney, 2010) as well as a deficiency of the hormone melatonin, produced at night, that regulates and suppresses production of estrogen (Mihu, 2009; compare Fig. 1). Without production of melatonin, excess estrogen may be produced, as a known tumor promoter and a vital contributor to

onset of breast and ovarian cancer (Anderson, 2002; Mihu, 2009; Merklinger-Gruchala, 2008). A similar correlation with night shifts has also been shown for ovarian cancer, but there was suggestive evidence of a decreased risk for "night people", or "owls", compared to "morning people", or "larks" (Bhatti et al., 2013). In a separate study, Lim and Saper (2012) recently reported that a single nucleotide polymorphism is correlated with whether someone is a "lark" or an "owl"; A-A genotypes woke up about an hour earlier than G-G genotypes and A-G genotypes woke up almost exactly in the middle (Lim and Saper, 2012). The latter authors also found that this genotype variation is also correlated with time of death. I suggest that the results of Bhatti (2013), showing an increased risk for ovarian cancer for those who have worked night shifts, but a lower increased risk for "night people" might be explained by a protective genetic interaction between genes promoting breast and ovarian cancer, such as disruption in the circadian rhythm genes and melatonin production, and this nucleotide variation identified by Lim and Saper (2012).

4.4. Interaction of BRCA with Other Genes

Henderson and Feigelson (2000) conclude in their review that breast cancer is a polygenic disease involving multiple genes in estrogen synthesis and estrogen receptor binding. Ovarian cancer is also thought to be polygenic, with an involvement of genes affecting FSH (follicle-stimulating hormone) and progesterone levels, both being hormones that function in regulation of the menstrual cycle and ovulation (Henderson and Feigelson, 2000). Multiple genes are thus responsible for hereditary breast and ovarian cancer (Boulton, 2006; Tan, 2008). However, the nature of these interactions is

poorly understood (Boulton, 2006). A specific example is the interaction with BRCA and insulin-like growth factor 1 (IGF-1, that, as is typical for growth factors, promotes cell division; Henningson, 2011). A link between increased IFG-1 and BRCA mutation tumors has been proposed (Hudelist, 2007; Pasanisi, 2011; Henningson, 2011). Henningson (2011) shows that the normal (un-mutated) BRCA gene suppresses the IGF-1 promoter, while a faulty BRCA is associated with greater IGF-1 expression. In addition, presence of a rare variant of IFG-1, associated with higher IGF-1 levels, in BRCA mutation carriers was associated with a younger diagnosis age (Henningson, 2011).

Knowledge of gene interactions, such as that between BRCA and IGF-1, can be important to BCRA studies; Pasanisi (2011) suggests that BRCA interaction with IFG-1 modulates penetrance level for BRCA mutation carriers. The proposed interaction between the sleep variants and breast and ovarian promoting genes above is another example that warrants further attention. For populations with genetic variants that increase or decrease the risk for breast and ovarian cancer, whether or not they involve BRCA, the causes of these differences in penetrance levels thus needs to be further addressed. Conversely, knowledge of prevalence and penetrance differences may aid in the discovery of protective or multiplicative genes. Moreover, identification of possible additional genetic interactions should further increase treatment options for patients.

5. Future Suggested Studies and Areas For Focus in BRCA Research

5. 1. Molecular Diagnostics

Molecular diagnostics in cancer research assesses an individual's genes, identifies specific mutations (among the many possible mutations, see below) harbored by this individual, and uses this information as a tool for cancer treatment and, possibly, prevention (Kalia, 2013). Currently, this work is only done as part of research projects and early phase clinical trials.

For a cancer tumor to form, not just one but multiple mutations must be accrued (Hanahan and Weinberg, 2000). Each of these mutations must affect components of key pathways controlling e.g. cell division, cell death, and DNA repair in the cell cycle to overcome the genome's naturally protective efforts. Because individual tumors possess different combinations of genetic mutations, each tumor responds differently to current standard cancer treatments (v'ant, 2002; Sim, 2013). Knowledge of these differences is needed to prevent and treat cancer. Genotyping, or the sequencing of the specific DNA makeup of individual tumors for the exact mutations involved, promises to identify the most effective cancer treatments. In turn, such individualized cancer treatments should reduce (i) health care cost, (ii) lost time due to ineffective treatments, (iii) unnecessary damage to non-cancer cells, and (iv) psychological stress from unproductive therapies.

The estrogen receptor status of breast cancer is a good example of the promise of molecular diagnostics. Identifying a breast tumor's presence and numbers of receptors for estrogen, progesterone and human epidermal growth factor type 2 can guide in selecting appropriate chemotherapies targeting these receptors.

Furthermore, I suggest using molecular diagnostics as a possible preventative tool. For individuals with inherited susceptibility mutations, such as BRCA mutations, one of the main mutations of the cancer is already known. Once the molecular pathways disrupted by the onset of cancer are known, a test for components of this pathway may allow early cancer detection.

Due to the promise of such research, I suggest that more focus be placed on molecular diagnostics in cancer research and in BRCA-specific research in the future.

5.2. Gene Therapy

Gene therapy involves introducing genes into an organism that will get taken up into the genome and be expressed (Niazi, 1997). Research on gene therapy to address cancer is focused on (i) replacing missing or altered genes with functional, "working" genes (e.g. tumor suppressor genes like BRCA 1 and 2, and others like p53; Hanahan and Weinberg, 2000; El-Aneed, 2004), (ii) improving a patient's immune response to cancer (by stimulating the immune system's natural ability to attack cancer cells), (iii) insertion of genes into cancer cells to make the latter more sensitive to chemotherapy, radiation therapy, or other treatments, (iv) introduction of "suicide genes" into a patient's cancer cells (a "pro-drug", as an inactive form of a toxic drug, is given and is activated specifically in cancer cells containing "suicide genes", "which leads to the destruction of those cancer cells" (El-Aneed, 2004), and (v) preventing cancer cells from developing new blood vessels (cancer cells exhibit prolific vascularization, or angiogenesis, and stop growing if blood supply is cut off; Naumov et al., 2006).

One of the current problems with gene therapy is the difficulty with inserting genes into their correct location in the human genome (Fey, 1999; El-Aneed, 2004). Genes have to first be introduced into a host via viral vectors, such as retroviruses, adenoviruses, adeno-associated viruses or lentiviruses (El-Aneed, 2004), but despite successful introductions of genes into these viral vectors and of vectors into the host, insertion of the gene in the proper location in the genome has been elusive (El-Aneed, 2004). Insertion in the wrong location has itself been associated with cancer – presumably since insertion into the wrong place in the genome can trigger oncogenes or suppress tumor suppressor genes (Baum, 2007). Clearly, more effective techniques are needed to deliver genes to specific target cells and locations in the genome.

Concerning gene therapy with BRCA genes, much of the available work was done by the group of Tait in the late 1990s and early 2000s (Tait, 1998,1999, 2000) in search of a vector allowing insertion of a functional (wild type) BRCA1 gene into mouse models. In 1998, retroviral-mediated delivery of BRCA1 gene therapy used in mouse models (Tait, 1998) was found to be effective in reducing tumor burden and to be minimally toxic. Subsequently, twelve human patients with recurrent or persistent ovarian cancer and participating in phase-I clinical trial were treated with one to three cycles of intraperitoneal (in the abdomen's peritoneal cavity) vector treatment of BRCA1 (Tait, 1998). Toxicity was minimal, and the vector was fairly stable and expressed in patient tissues (Tait, 1998). Stabilization of the disease, or absence of tumor growth, was noticed in eight of the 12 patients, suggesting that the peritoneal cavity may be an appropriate site for gene therapy (Tait, 1998). The latter study proved not only that BRCA1 was successful in a vector, but also that the peritoneal cavity is a promising

candidate for future treatments. Finding an effective site for vector delivery is important in the effectiveness of the treatment. However, the effectiveness of this vector diminished in phase II trials, such that there was no disease stabilization, and little or no vector stability (Tait, 1998).

The same group (Tait, 2000) subsequently used MFG-BRCA1, a new retroviral BRCA1 vector. Vector stability studies were performed with mice and human serum *in vitro* (in a Petri dish), mice were subsequently injected intraperitoneally with ovarian cancer cells, and tumors were allowed to grow for 4 weeks, after which mice were treated intraperitoneally with either MFG-BRCA1 or control vectors (Tait, 2000). Survival was three-fold greater in these treatments compared to control vectors, and the latter vector was more stable than the previously used LXSN-BRCA1sv vector (Tait, 2000).

Laura Vannucci and coworkers (2010) used a lentiviral (retrovirus) vector based on the feline (cat) immunodeficiency virus (FIV) to import functional, wild type BRCA1 into human mammary cells with a non-functional BRCA1 gene *in vitro*. This FIV viral vector was used because there are no documented cases of FIV infecting humans, unlike HIV (Vannucci, 2010). Lentiviruses are also preferable for gene therapy due to their ability to integrate in a directed way into the host genome (causing decreased cancer risk from improper insertion), and to ensure long-lasting expression of the transplanted genes due to their ability to implant into non-dividing cells (Lever, 2004). Other viruses and retroviruses currently used for gene therapy are less effective at these aforementioned functions (Lever, 2004). A human epithelial (293T) tumor cell line (obtained from a 24-year old patient with primary breast cancer and carrying a germ-line mutation in BRCA1) was used for the *in vitro* tests (Vannucci, 2010). Cells showed fully functional BRCA1

gene over a period for one month of observation (observation stopped after one month); BRCA1-transduced cells repaired DNA double strand breaks more efficiently and grew five times faster compared to control cells (Vannucci, 2010). Overall, the latter study shows that functional, wild type BRCA1, delivered and expressed into defective cells bearing an inactive BRCA1 gene, restores normal gene function and contributes to maintaining cell homeostasis in human epithelial cells *in vitro*.

The promise of gene therapy to restore non-functional BRCA cells to functional cells is thus considerable. Because of the encouraging developments in this area of research, I suggest gene therapy as a continued area for focus research on cancer and BRCA mutations. Furthermore, the use of germ line gene therapy to integrate a functional gene into the germ line and therefore eradicate mutations from subsequent generations, is a particularly promising area for future research.

6. Conclusions

In conclusion, more BRCA testing, and more testing of random individuals (beyond families with a known elevated cancer risk), needs to be conducted. BRCA mutations are found worldwide, but more testing needs to be done in order to accurately assess which populations are at higher risk for presence of the faulty gene, and what the reasons are for differences in penetrance. Second, BRCA1 and 2 need to be analyzed separately, due to their differing characteristics, such as presence or absence of estrogen receptors. The latter two genes need to be analyzed separately especially in all studies of factors with a possible connection to estrogen levels. Third, studies on the effects of lifestyle factors need to include information on exposure to these factors for many years

prior to the cancer diagnosis and need to assess synergistic effects of dietary/lifestyle factors. Fourth, molecular diagnostics and gene therapy – as showing great promise for the treatment and prevention of cancer – should be a key focus of future research. Since research progress appears to be hindered by a fracturing of the field into many different disciplines, an effort to integrate these multiple disciplines towards more synergistic efforts should make research easier, faster and more effective. Additionally, immediate suggestions for those with BRCA mutations to lower cancer risk are to follow a diet rich and diverse in fruits, vegetables and phytochemicals and low in alcohol, maintain a low Body Mass Index, use oral contraceptives for ovarian cancer risk reduction, and get enough sleep with a reduction in night activity.

7. References

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