Chapter 2
Alcohol and Breast Cancer: Reconciling Epidemiological and Molecular Data

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Abstract Breast cancer is the most diagnosed cancer in women worldwide. Epidemiological studies have suggested a possible causative role of alcohol consumption as a risk factor for breast cancer. However, such conclusions should be interpreted with considerable caution for several reasons. While epidemiological studies can help identify the roots of health problems and disease incidence in a community, they are by necessity associative and cannot determine cause and effect relationships. In addition, all these studies rely on self-reporting to determine the amount and type of alcoholic beverage consumed, which introduces recall bias. This is documented in a recent study which stated that the apparent increased risk of cancer among light-moderate drinkers may be "substantially due to underreporting of intake." Another meta-analysis about alcohol and breast cancer declared "the modest size of the association and variation in results across studies leave the causal role of alcohol in question." Furthermore, breast cancer develops over decades; thus, correlations between alcohol consumption and breast cancer cannot be determined in epidemiological studies with windows of alcohol exposure that captures current or recent alcohol intake, after clinical diagnosis.

Numerous risk factors are involved in breast carcinogenesis; some are genetic and beyond the control of a woman; others are influenced by lifestyle factors. Breast cancer is a heterogeneous and polygenic disease which is further influenced by epigenetic mechanisms that affect the transciptomes, proteomes and metabolomes, and ultimately breast cancer evolution. Environmental factors add another layer of complexity by their interactions with the susceptibility genes for breast cancer and metabolic diseases. The current state-of-knowledge about alcohol and breast cancer association is ambiguous and confusing to both a woman and her physician.

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Confronting the huge global breast cancer issue should be addressed by sound science.

It is advised that women with or without a high risk for breast cancer should avoid overconsumption of alcohol and should consult with their physician about risk factors involved in breast cancer. Since studies associating moderate alcohol consumption and breast cancer are contradictory, a woman and her physician should weigh the risks and benefits of moderate alcohol consumption.

Keywords Breast cancer • Epidemiology • Alcohol • Acetaldehyde • Reactive oxygen species • Estrogen • Folate • Metabolism • Epigenetics • Alcohol dehydrogenase • Aldehyde dehydrogenase • BRCA1 • BRCA2

2.1 Introduction

Cancer is the leading cause of death in developed countries; worldwide, it is estimated that cancer could result in 12 million deaths in 2030 [1]. The most common cancers worldwide are lung, breast, colorectal, stomach, and prostate. In women, the leading causes of cancer death are lung, breast, and colorectal cancers. In an annual report by the National Cancer Institute [2], overall cancer death rates continued to decrease in the USA in the period between 1975 and 2010; most declines were observed in female breast, prostate, lung, and colorectal cancers. The sharp decrease in breast cancer between 2002 and 2003 was attributed most likely to the reductions in the use of postmenopausal hormone-replacement therapy (HRT) [3]. Notwithstanding the significant decline in breast cancer mortality rates in the industrialized nations since 1990, breast cancer represents the most common female malignancy worldwide and is one of the primary causes of death among women globally [4].

There are a multitude of underlying etiological risk factors for breast cancer, enumerated below, including the use of HRT. However, before discussing risk factors, it is imperative to understand how breast cancer develops.

2.2 The Biology of Breast Cancer

Breast development starts by the rapid division of stem cells at puberty and continues through woman's first full-term pregnancy. After birth, the hormonal milieu (estrogen, progesterone, growth hormone, prolactin) and cell fate-determining signaling pathways transform a high percentage of mother's breast cells into mature, differentiated milk-producing cells. Breast cell division is controlled by signals, such as estrogen, that allow cells to enter the cell cycle and promote cell division. Many proto-oncogenes code for the signals that control the cell cycle. Certain mutations in proto-oncogenes can result in oncogenes that code for protein

signals that cause overexpression of growth factors or their receptors, resulting in uncontrolled cell division and growth. For instance, erbB2, a member of the epidermal growth factor (EGF) receptor family, also known as HER-2 (for Human Epidermal Growth Factor Receptor 2) or HER2/neu is a receptor tyrosine kinase protein that promotes cell proliferation. HER2 itself does not bind growth factors, but it can heterodimerize with other members of the EGF receptor family and channel EGF and growth factor signals into more effective growth-promoting pathways. Overexpression of HER2 can thereby enhance the growth and proliferation of cancer cells; HER-2-positive breast cancers are more aggressive than other types of breast cancer. Other oncogenes that influence breast cancer include many other members of the tyrosine kinase family as well as cell cycle regulatory proteins, such as c-myc, cyclin D-1, and the cyclin regulator CDK-1. Opposing the oncogenes are tumor suppressor genes, such as p53 which recognizes cells with mutated DNA and causes apoptosis to these cells. Mutations in the p53 gene result in the continuous reproduction of cells with damaged DNA, enhancing cancer development [5].

Breast cancer is a heterogeneous disease that encompasses more than 20 different subtypes. Numerous molecular, cellular, and pathological processes are involved in the transformation of healthy tissue to preinvasive lesions, such as ductal carcinoma in situ (DCIS), to invasive breast cancer. More than 70 % of DCIS lesions express estrogen receptors, and about 50 % of the lesions overexpress the *HER2/neu* proto-oncogene [6], In addition, the p53 tumor suppressor gene is mutated in roughly 25 % of lesions [7]. Based on molecular characteristics and clinical outcome, subtypes of breast cancer are defined by gene expression profiles including evaluation of estrogen receptor (ER), progesterone receptor (PR), and HER2 receptor, all of which affect the tumor growth rate and its metastatic potential, reflected in the disease grading [8, 9].

In addition to ER and PR, studies have revealed the presence and potential importance of several nuclear receptors in breast cancer, including receptors for steroid hormones (androgen, corticosteroids), vitamins A and D, fatty acids, and food-derived xenobiotic lipids [10]. Among other major signaling pathways involved in mammary carcinogenesis is increased Wnt signaling [11]. The Wnt signaling pathway controls the stability and activity of β-catenin, a transcription factor that drives the expression of a large number of proliferation promoting signals, as well as signaling pathways that control the activity of mTOR, a critical junction in the cell-growth control. Wnt signaling is an important factor during mammary development and is involved in stem cell fate determination. Wnt also determines the differentiation of cancer stem cells, and its unregulated activation can promote tumorigenesis. In particular, there is evidence that Wnt activation is involved in triple-negative breast tumors, i.e., breast tumors that are not characterized by overexpression of HER2, ER, or PR. The role of aberrant Wnt signaling in breast carcinogenesis is further highlighted by the finding that knockdown of the tumor-suppressor gene PTEN (*phosphatase and tensin homolog deleted on chromosome 10*) resulted in the activation of the Wnt/β-catenin pathway in human breast cells [12]. In addition to Wnt signaling, notch signaling regulates mammary stem and progenitor cell activity in breast tissue and commits stem cells to the luminal cell lineage [13].

2.3 Known Risk Factors for Breast Cancer

To fully understand the findings and ramifications of epidemiological studies on alcohol and breast cancer, it is essential to consider the range of known risk factors involved in breast cancer development. Many of the primary risk factors for breast cancer cannot be readily modified. These include the strong risk factors aging, genetics (inherited changes in certain genes and family history of breast cancer), risk caused by prenatal history (e.g., daughters born to mothers who used diethylstilbestrol (DES) during pregnancy have increased risk), and reproductive parameters which determine the cumulative lifetime estrogen exposure (early menarche, before age 12; delayed menopause, after age 55; delayed child bearing; first full-term pregnancy after age 30; miscarriage; abortion). Modifiable lifestyle risk factors include dietary habits (consumption of polyunsaturated fats and excessive alcohol), smoking, exposure to radiation or synthetic estrogens, viral infection, physical inactivity, use of HRT, obesity, diabetes, breast implants, and even changes in circadian rhythm homeostasis, such as night-shift work. Needless to say, other risk factors may exist that are not yet fully understood or even known.

2.4 Alcohol as a Risk Factor for Breast Cancer

Chronic heavy alcohol consumption (drinking too much too often) and binge drinking (too much too fast) are risky drinking behaviors that could promote various pathological conditions, including cancer. More recently, some epidemiological studies have suggested that even moderate alcohol consumption can increase the risk of breast cancer by a small extent [14]. By contrast, others reported a decrease in breast cancer risk due to moderate drinking [15]. Equally, the molecular basis of alcohol use as a risk factor remains disputed. In view of the contradictory results of the epidemiological as well as the molecular studies on alcohol and breast cancer, the landscape of available information will be discussed under these two categorizations: (a) *epidemiological studies*, which cover case–control or cohort studies, conducted in various countries and with a wide range of cohort size, and (b) studies addressing the *molecular basis* that might contribute to the influence of alcohol on breast cancer risk. We will then consider to what extent information on molecular and cellular actions of alcohol can account for the epidemiological findings on alcohol as a risk factor for breast cancer.

2.4.1 Epidemiological Studies

A large number of prospective studies and some case–control studies on alcohol use as a risk factor for breast cancer have been reported over the past decades. Although there is consensus that heavy alcohol use can be a significant risk factor, the findings

are more controversial with regard to moderate alcohol use. These studies use a wide range of different sample sizes and methodologies, various definitions of a "drink," and diverse criteria of moderate or heavy drinking and consider different times of drinking in a woman's life. A constant feature is that essentially all studies obtain alcohol use data by self-reporting, the reliability of which is often problematic, particularly for longer time intervals. All these factors can explain at least part of the divergent findings and confusion. Studies discussed below are not intended to present a comprehensive review of epidemiological studies, rather a sampling of various studies, in different countries, with diverse methodologies and sample size, varied dietary intake, and different results. For clarity of the discussion that will follow, these studies are enumerated below, and combined comments on them are discussed in the concluding remarks section.

1. The Nurses' Health Study initiated in the USA in 1980 administered a dietary questionnaire (including the use of beer, wine, and spirits) to 89,538 nurses between the age of 34 and 59, with no history of cancer. During the ensuing 4 years, 601 cases of breast cancer were diagnosed. (In this study, a drink was defined by rather nonstandard criteria with inaccurate estimates of their alcohol content). The study reported relative risk (RR) of 1.3 for women consuming one-third to one drink/day (compared to nondrinkers RR = 1.0), which went up to 1.6 for those consuming more than one drink/day, although, ironically, RR was not further increased in those consuming 1.8 or more drinks/day, and there was no increase in risk for those who drank less than 1/3 of a drink/day [16]. The study noted the potential impact of various other risk factors, such as body weight, cigarette smoking, and being nulliparous, but stated that these were not themselves associated with breast cancer risk in these studies. However, several other studies have reported that these are risk factors for breast cancer (see below). Moreover, the combined risk of alcohol use with these other factors could not be resolved.
2. An update of the Nurses' Health Study (1) was published in 2011 [17]. Cumulative average alcohol intake in 1994, the midpoint of the follow-up period, was used to assess RR for breast cancer. Compared with women who never consumed alcohol, those who consumed 5–9.9 g per day (equivalent to 3–6 drinks per week) had a modest increase in risk (RR = 1.15); little difference was found between risk and various alcoholic drinks (RR per 10 g/day was 1.12 for wine and 1.09 for beer or liquor). Women who on average consumed at least 30 g of alcohol/day (slightly over two drinks per day) had a greater risk of breast cancer (RR = 1.51). Alcohol consumption seemed to be more strongly associated with the risk of ER+ status, PR+ status, or both for women who drank 10 or more g/day; this interaction did not reach statistical significance though. The authors of this study highlighted the importance of considering lifetime exposure when evaluating the effect of alcohol. However, determining lifetime alcohol use by average use/day may miss important patterns of alcohol use that may have an influence on the outcome, as was recognized in some other studies.

3. In 1994, Longnecker [18] reported on a meta-analysis of 29 case–control and 9 follow-up studies from the USA, Australia, Italy, France, Greece, the Netherlands, Canada, England, Sweden/Norway, Denmark, New Zealand, and Argentina. Daily consumption of a drink was associated with an 11 % increase in breast cancer risk compared to nondrinkers. However, the author reported that the "slope of the dose response curve was quite modest" and "the modest size of the association and variation in results across studies leave the causal role of alcohol in question." Needless to say, these studies were conducted in different countries with wide variations in their dietary habits, environmental factors, smoking, and genetic background.
4. In a case–control study of 890 cases of Black and White women, 20–74 years old, in the USA, the odds ratio (OR) to develop breast cancer for women who have a recent consumption of 1 or 2 drinks/day, compared to nondrinkers, was 1.4; intriguingly, consumption of two or more drinks/day resulted in OR of 1.0 (i.e., no increase in risk). In addition, average lifetime consumption of 91 g/week (about 6.5 drinks) resulted in a "nonsignificant increased" risk (OR = 1.5) in women reporting binge drinking [19]. Also, ORs did not differ by race, age, menopausal status, use of HRT, or body mass index (BMI). Obviously, these correlations are intrinsically questionable. It is hard to see how recent consumption can be a causal factor in breast cancer that probably has started at least 20 years earlier. Also, it is difficult to correlate average lifetime consumption in a meaningful manner with the molecular events leading to breast cancer.
5. A small case–control study in France involving 437 women between the age of 25 and 85, reported a decrease in risk for breast cancer for women consuming less than 1.5 drinks/day; OR = 0.58, after adjustment for BMI, parity, breastfeeding, physical activity, history of breast cancer, diet, and duration of ovulation [20]. Three patterns of alcohol consumption were identified (abstinent, sporadic, and frequent drinkers). Sporadic drinkers comprised women who drank four times per week or less, while frequent drinkers were defined as those who consumed alcohol five times a week or more. Alcohol consumption was recorded as units (one unit = 10 g of ethanol in 4.2 oz of wine, 11 oz of beer, or 1 oz of spirits). No association was found between the pattern of total alcohol consumption and breast cancer risk. The study noted that drinking pattern could change during the period under consideration. For example, "a woman who claimed not to be drinking at the time of interview could, in fact, have been at some previous point alcoholic or could have had sporadic alcoholism that motivated the cessation of drinking. In such cases, the longest typical phase of consumption during that individual's history was used for the study."
6. The risk of breast cancer due to total caloric intake, coffee and alcohol consumption was studied in 280 breast cancer French Canadian women who were noncarriers of six specific mutations in *BRCA1/2* genes found more frequently in families of French Canadian descent. They were compared with 280 matching women without breast cancers who were not carriers of these mutations [21]. Data were obtained by using a food-frequency questionnaire (FFQ) that

"covered the period 2 years prior to the diagnosis for cases and a corresponding period for the controls." In addition, "alcohol-related beverages consumed were summed to obtain the total amount drunk per week." Average alcohol consumption was 9.8 and 6.3 g/day for cases and controls, respectively. The study concluded that "more than two bottles of beer per week" increased breast cancer risk by 34 %, whereas >10 oz of wine or >6 oz of spirit per week increased cancer risk by 16 % and 9 %, respectively. The study acknowledged "recall bias" as a limitation.

7. A case–control study involving 1,728 women 20–49 years of age, in Los Angeles County, California, administered a questionnaire about early, lifetime, or recent alcohol consumption [22]. The study reported that alcohol intake "during the recent 5 year period before the breast cancer diagnosis was associated with increased breast cancer risk" and that "intake of two or more alcoholic drinks per day during this 5 year period was associated with an 82 % increase in breast cancer risk relative to never drinkers." Ironically, there was no risk increase for "lifetime alcohol intake."
8. On the other hand, a population-based study (1,508 cases) collected information on alcohol intake throughout life. Consumption of 15–30 g/day (approximately 1–2 drinks) throughout life was associated with a modest 33 % increase in risk particularly among women with low BMI (<25) and those diagnosed with estrogen receptor-positive tumors; but heavier consumption (>30 g per day) was not. Risk did not vary with alcohol type or by patterns of use (recent use, intake prior to age 20 years) [23].
9. Another population-based case–control study about lifetime alcohol consumption did not find an increase in breast cancer risk among women younger than 50 years of age; however, among those over 50 years of age, ever drinking conferred a relative risk of 1.8. Information about alcohol intake was obtained using a questionnaire from women 40–75 years old who participated in a screening program in central Sweden [24].
10. Breast density is a risk factor for breast cancer. The impact of alcohol consumption on mammographic density was assessed for 1,207 cases from three populations (Japan, Hawaii, California) [25]; alcohol intake was estimated from "self-administered questionnaire" and recorded as "ever vs. never," and for Hawaii and Japan only, the "ever drinkers were divided into ≤1 and >1 drink/day." Results showed that alcohol consumption did not significantly modify the effect of mammographic density on breast cancer risk "in this pooled analysis." The study stated that "whereas the dichotomous model did not indicate an association between alcohol drinking and breast cancer, the relative risk was elevated for women consuming >1 drink/day without reaching statistical significance." The study invoked "recall bias" and stated that "as in all epidemiological studies, alcohol intake may have been underreported" and "this analysis had limited ability to model the exact relations between alcohol intake, mammographic density, and breast cancer risk and the findings need to be interpreted with caution."

In short, these various studies highlight the intrinsic problem of assessing long-term or even lifetime drinking patterns through a recall questionnaire approach.

Another question is whether specific subtypes of breast cancer show enhanced risk related to past or current alcohol use and whether alcohol use synergizes with other breast cancer risk factors. In recent years, a number of large cohort studies were conducted that provided the opportunity to assess alcohol use history and other breast cancer risk factors for some of the major cancer subtypes.

11. The Million Women Study [26] conducted in the United Kingdom and published in 2009 calculated the RR for 21 site-specific cancers due to beverage alcohol consumption, including breast cancer, based on a questionnaire asking about the average alcohol consumption per week. Of the 1,280,296 women recruited, data from 708,265 women from a follow-up survey three years later were used. The study reported that women who drank alcohol were "likely to be younger, leaner, more affluent, and to do strenuous exercise more frequently" and more likely to "have ever used oral contraceptives and to be currently using hormone replacement therapy" than nondrinkers. Also, among drinkers, "the proportion of current smokers increased with increasing alcohol intake." The RR of breast cancer was 1.08, 1.13, and 1.29 for women who drank 3–6, 7–14, and 15 or more drinks/week, respectively. The study estimated a 12 % increase in breast cancer risk per 10 g increment of alcohol intake.
12. The Women's Health Study in the USA conducted a 10-years follow-up on 38,454 women 45 years or older who were free of cancer and cardiovascular disease at baseline and provided detailed dietary information, including alcohol consumption [27]. High alcohol consumption (30 g/day—over two drinks) was associated with a modest increase in breast cancer risk (RR = 1.32) that was limited to ER+ and PR+ tumors. The RR for an increment of 10 g/day of alcohol were 1.11 for ER+/PR+ tumors, 1.00 for ER+/PR– tumors, and 0.99 for ER–/PR– tumors. The association seemed strongest among those taking HRT currently, albeit statistically not significant. In addition, the RR of breast cancer for a 10 g/day increment was similar for different beverages (1.15 for beer, 1.13 for white wine, and 1.08 for red wine or liquor).
13. In a population-based Swedish Mammography Cohort study, self-reported data on alcohol consumption were collected in 1987 and 1997 from 51,847 postmenopausal women [28]. After adjusting for age; family history of breast cancer; BMI; parity; age at menarche, first birth, and menopause; diet; and HRT use, alcohol consumption was associated with an increased risk for the development of ER+ tumors, irrespective of PR status, especially in women using HRT. Consumption of 10 g or more of alcohol/day increased RR to 1.35 for ER+/PR+, 2.36 for ER+/PR−, 0.62 for ER−/PR+, and 0.80 for ER−/PR− tumors versus nondrinkers. However, the link between alcohol use and ER+ or PR+ status was not consistent across different studies. The study by Terry et al. [23] mentioned earlier reported that alcohol consumption increases the risk in ER+/PR+ breast cancer but not in ER−/PR− [23]; among postmenopausal

women no statistically significant differences were observed in the risk factor profiles for ER+ PR+ and ER−PR− breast cancer [29]. An additional case–control study showed that alcohol increases risk in ER+/PR+ tumors, but not for ER+/PR− and ER−/PR− tumors [30]. Alcohol use appears to be more strongly associated with risk of lobular carcinomas and hormone receptor-positive tumors than it is with other types of breast cancer [31]. To add to the confusion, another study reported an increased risk for ER+/PR+, ER−/PR−, and ER−/PR+ tumors, but not for ER+/PR− in women 20–44 years of age [32]. Two additional case–control studies reported positive associations of alcohol consumption with risk of either ER+ or ER− tumors [33, 34] and one with ER+ tumors only [35]. Finally, two studies in which alcohol consumption was categorized into only "ever vs. never" reported no association irrespective of joint ER and PR status [36, 37].

14. In contrast to the Swedish study, the Iowa Women's Health Study found that alcohol intake was most strongly associated with ER–/PR– tumors in following 37,105 cancer-free women 55–69 years of age, who filled out a questionnaire by mail, and were followed up for 7 years [38]. Alcohol consumption over the past year was self-reported and was averaged as g/week. The study reported that there was a 55 % increased risk for ER−/PR− tumors in "women who had ever drunk alcohol"; however, alcohol consumption was not quantified.
15. The interactions between alcohol consumption and HRT was studied in 40,680 postmenopausal California teachers using a questionnaire for alcohol consumption during the past year and HRT use for the past 5 years [39]. Subjects are grouped into three categories: nondrinkers, those consuming <20 g/day of alcohol, and those who consume ≥20 g/day. Increased breast cancer risk associated with alcohol consumption was observed among postmenopausal women who were current users of HRT (RR = 1.60 for those consuming <20 g/day and RR = 2.11 for consumers of ≥20 g/day). Alcohol did not increase risk among women who had stopped using HRT within 3 years. Results were similar for ER+ and ER+/PR+, and no increase in risk was observed in ER− tumors.
16. A study on 989 cases of breast cancer in women aged 23–74 years in three Italian areas investigated the role of alcohol according to ER and PR status by collecting information on lifetime alcohol consumption using FFQ [40]. The weekly number of drinks was calculated, taking into account that one drink corresponds to approximately 125 mL of wine, 330 mL of beer, and 30 mL of hard liquor, each containing about 15 g of ethanol (30 mL of 80 proof liquor contains only 9.6 g of ethanol). The study reported that consumption of ≥13.8 g/day increased the risk of ER+ tumors (OR = 2.16), ER− (OR = 1.36), ER+/PR+ (OR = 2.34), ER−/PR− (OR = 1.25) and concluded that alcohol is more strongly associated with ER+ and ER+/PR+ than ER− breast tumors.
17. The National Institutes of Health-AARP Diet and Health Study obtained information from 184,418 postmenopausal women aged 50–71 years, about their alcohol use and diet, through a mailed questionnaire at baseline [41], Breast cancer cases and ER and PR status were identified through linkage to state cancer registries. The authors reported that "Moderate consumption of alcohol

was associated with breast cancer, especially hormone receptor-positive tumors." However, a closer analysis of the data indicated that the RR did not reach significance for both light (0.4–0.7 self-reported drinks/day) and moderate (0.7–1.4 drinks/day) alcohol use (RR of 1.13 and 1.07, respectively, for ER+/PR+ cancers, with 95 % confidence intervals ranging from 0.89 to 1.38) and even self-reported drinking at higher levels (1.4–2.5 drinks/day) with an RR of 1.34 and CI 1.06–1.69 was based on only 89 cases. Other cancer subtypes (ER+/PR− or ER−/PR−) did not show significant increases and were based on even lower incidence. Therefore, the conclusion of the authors that moderate drinking was associated with breast cancer is not supported by data.

18. The Women's Health Initiative-Observational Study enrolled 87,724 postmenopausal women aged 50−79 years, without a history of breast cancer between 1993 and 1998, who self-reported their alcohol use histories [42]. In a follow-up through 2005, a total of 2,944 patients with invasive breast cancer were diagnosed. The study reported that the RR in women who consumed seven or more alcoholic beverages/week was 1.82 for hormone receptor-positive invasive lobular carcinoma and a statistically nonsignificant 1.14 for hormone receptor-positive invasive ductal carcinoma. Women who reported drinking one or more alcoholic beverage/day were more likely to be nulliparous, with low BMI, currently use HRT, and smoke. Alcohol use was assessed only at baseline, and the authors stated that "Extensive measurement errors or changes in alcohol use could affect the study conclusions."
19. In 1966, Doll and colleagues reported on breast cancer incidence in five continents where the USA was reported to be 4–7 times higher than in Asian populations [43]. Almost half of the East Asian population is deficient in the mitochondrial enzyme that metabolizes acetaldehyde, the first metabolite of alcohol and a suspect in breast carcinogenesis. Although the drinking history of breast cancer patients was not assessed in this study, this observation suggests that acetaldehyde metabolism may not be a dominant determinant in breast cancer risk. To test any association between acetaldehyde and breast cancer, the effect of alcohol consumption on breast cancer incidence rates was studied in 597 Chinese, Japanese, and Filipino women living in San Francisco–Oakland, Los Angeles, and Oahu, Hawaii [44]. Breast cancer risk was not significantly associated with alcohol drinking (OR = 0.9) in Asian American women. Furthermore, a prospective study performed in Japan using data from 35,844 women who completed a self-administered questionnaire found that consuming <15 g/day did not significantly increase the risk for breast cancer. However, risk was significantly increased in women who consumed ≥15 g/day [45]. To add to the confusion, the Miyagi Cohort Study in Japan involving 19,227 women found that consuming ≥15 g/day of alcohol "had no significant relation to breast cancer risk" [46].
20. To test whether alcohol-induced facial flushing (i.e., women who have the defective ALDH2*2 gene thereby cannot effectively metabolize acetaldehyde further to acetate) modifies the risk for breast cancer, a prospective study was undertaken by Japan Public Health Center on 50,757 pre- and postmenopausal

women aged 40–69 years, using self-reported questionnaire [47]. After 13.8 years of follow-up, 572 cases of invasive breast cancer were diagnosed. The study reported that, compared to never drinkers, regular alcohol drinkers (>150 g ethanol/week—about 2 drinks/day, which is higher than the definition of moderate drinking) have 78 % increased risk for breast cancer in premenopausal women and 21 % increase in postmenopausal women. Consumption of 10 g/day of alcohol was associated with 6 % increase in risk for overall breast cancer (compared to 12 % in the Million Women Study discussed above). This effect was not modified by alcohol-induced facial flushing, by folate intake, by smoking, by BMI, nor by exogenous estrogen use by postmenopausal women. There was no statistically significant association between alcohol intake and ER+ tumors. A previous study also showed no association between polymorphism of ALDH enzyme and risk of breast cancer [48]. Furthermore, a review of epidemiological evidence in Japanese populations using three cohort studies and eight case–control studies by Nagata and colleagues reported that "epidemiologic evidence on the association between alcohol drinking and breast cancer remains insufficient in terms of both the number and methodological quality of studies among the Japanese population" [49].

21. A case–control study conducted in China involved 1,009 cancer cases, in which alcohol consumption data were obtained in a face-to-face interview within three months after diagnosis. Tumors' ER/PR status was obtained from pathology reports. The study reported that low-moderate alcohol consumption was inversely associated with breast cancer risk: adjusted odds ratio (OR) for women who consumed <5 g/day was 0.4 and 0.62 in post- and premenopausal women, respectively, compared to nondrinkers [15]. OR was low across hormone receptor status groups even for those consuming <15 g/day for postmenopausal women (OR = 0.36–0.56) and premenopausal women (OR = 0.57–0.64). Consuming >15 g/day increased OR in postmenopausal women regardless of the hormone receptor status. Apart from the wide range of participants' age (20–87 years)—which influences the relative contribution of various risk factors for breast cancer-quantification of alcohol consumption was haphazard. For instance, the study stated that "Standard drinking vessels used by Zhejiang residents were displayed during the interview to increase the accuracy of measurement" without stating the volume or alcohol content. Furthermore, alcohol consumption was based on a "reference" recall period one year "before diagnosis." Consumption of ≥15 g/day appeared to increase breast cancer in postmenopausal women with ER+/PR− or ER−/PR+.
22. Similarly, a study on 712 breast cancer cases, aged 30–74 years from the New Mexico Tumor Registry, collected data on recent and past alcohol intake via in-person interview. Compared to nondrinkers, low recent alcohol intake (<148 g/week, ~10.5 drinks) was associated with reduced risk of breast cancer for non-Hispanic Whites (OR = 0.49) independent of hormone receptor status for both pre- (OR = 0.29) and postmenopausal women (OR = 0.56). Past alcohol intake did not demonstrate association with breast cancer, and trends were nonsignificant [50].

Several studies explored the relationship between alcohol use and folate deficiency as related risk factors in the development of breast cancer.

23. A study conducted on 1,000 Mexican women [51] with breast cancer using "in-person interviews" determined "recent alcohol intake" and whether the patient is an "ever drinker" or "never drinker." It concluded that "any alcohol intake increases risk of breast cancer," and "insufficient intake of folate may further elevate risk for developing breast cancer." "Ever drinking was associated with a twofold increase in the odds of breast cancer" reported the study. However, the definition of ever drinking was a "yes" or "no" without quantification, and the authors declared that "recall bias is a concern."
24. Another study, the Women's Health Initiative-Observational Study, gathered baseline questionnaires which addressed alcohol and folate intake from 88,530 postmenopausal women 50–79 years [52] and found no evidence for folate attenuating alcohol's effect on breast cancer risk. Similarly, the American Cancer Society Cancer Prevention Study II Nutrition Cohort [53] examined the relationship between alcohol, dietary intake of folate and methionine, and breast cancer risk in 66,561 postmenopausal women. Women who consumed 15 or more grams of ethanol/day had increased risk of breast cancer (RR = 1.26) compared with nonusers. However, no association between risk of breast cancer and dietary folate, total folate, or methionine intake was found, and there was no evidence of an interaction between dietary folate or total folate and alcohol.
25. Possible interaction between alcohol and folate was investigated in 24,697 postmenopausal women in the "Diet, Cancer and Health" follow-up study which included 388 cases of breast cancer and 388 randomly selected controls to estimate the breast cancer incidence rate ratio (IRR) in conditional logistic regression analysis [54]. Alcohol intake was associated with risk of breast cancer mainly among women with folate intake below 300 μg (IRR = 1.19 per 10 g average daily alcohol intake); no association between alcohol and breast cancer risk was found among women with a folate intake higher than 350 μg (e.g., folate intake >400 μg; IRR = 1.01). The authors concluded that adequate folate intake may attenuate the risk of breast cancer associated with high alcohol intake.
26. A case–control study in pre- and postmenopausal Japanese women including 1,754 breast cancer patients aged 20–79 years found that self-reported alcohol consumption was associated with the risk of breast cancer [55]. Consuming ≥23 g/day of alcohol increased the risk by 39 % compared to nondrinkers. However, no significant positive association was observed among premenopausal women. High folate intake was associated with a lower risk of developing breast cancer in pre- but not postmenopausal women. In addition, high folate intake reduced the risk of breast cancer in women consuming ≥23 g/day of alcohol only in post- but not premenopausal women. Determining the risk based on the tumor receptor status was misleading and confusing. For example, in premenopausal women with ER+/PR+/HER2+ tumors, the odds ratio (OR) of developing breast cancer for those drinking 1 to ≤5 g alcohol/day,

5 to ≤23 g/day, and ≥23 g/day were 0.84, 1.61, and 0.84, respectively. For ER−/PR−/HER2+ OR was 0.7, 1.92, and 0.52, respectively. Examination of data revealed that the ORs for ER−/PR−/HER2+ tumors were based on 4, 7, and 1 patients, respectively. Similarly, for ER−/PR−/HER2− tumors, ORs were 0.47, 2.47, and 1.39 based on 2, 5, and 1 patients, respectively.

27. The relation between alcohol intake and the risk of breast cancer was investigated in 274,688 women participating in the European Prospective Investigation into Cancer and Nutrition study (EPIC). Alcohol information was obtained by self-reports. The IRR per 10 g/day of continuous higher recent alcohol intake was 1.03. No association was seen between lifetime alcohol intake and risk of breast cancer. No difference in risk was shown between users and nonusers of HRT, and there was no significant interaction between alcohol intake and BMI, HRT, or dietary folate [56].

In summarizing the main outcomes of the epidemiological studies, despite the indications suggested by many of these studies that there is some relationship between alcohol use history and the risk for developing breast cancer, the nature of that relationship remains poorly characterized. Major open questions are what aspects of a woman's drinking history influence breast cancer risk, whether different subtypes of breast cancer account for the increased risk, and how an individual's physiological response to alcohol and its metabolites could interact with other breast cancer risk factors to promote disease onset or progression. A better understanding of the molecular basis by which alcohol use is thought to enhance cancer risk is needed. The following section will explore the information available from molecular and cellular studies that have addressed these questions.

2.4.2 Molecular Studies

Although epidemiological studies about alcohol and breast cancer resulted in controversial results, identified no causal association, and at low to moderate levels of drinking correlations were tenuous at best, experimental studies suggested possible mechanisms that could be invoked, including estrogen metabolism and response, acetaldehyde-induced cell mutation, oxidative stress, and epigenetic modifications involving one-carbon metabolism pathways. These mechanisms are elegantly reviewed by Seitz and colleagues [57] and by Dumitrescu and Shields [58].

2.4.2.1 Estrogen Metabolism

Estrogen plays an important role in breast cell division and hence carcinogenesis. It has been postulated that prolonged exposure of mammary tissue to estrogen and progesterone, due to early menarche and/or delayed menopause, may contribute to higher breast cancer risk. In postmenopausal women, estrogen levels are maintained

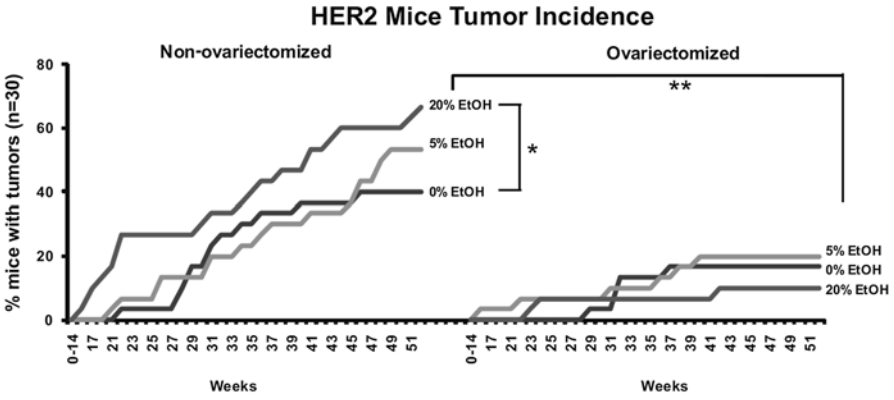

Fig. 2.1 Consumption of 20 % alcohol enhanced tumorigenesis in mice. Ovariectomy abolished this effect despite alcohol-induced increase in estrogen levels (From Wong et al. [61])

mostly by the activity of the aromatase enzyme which catalyzes the last step in estrogen biosynthesis from androgens (i.e., androstenedione to estrone and testosterone to estradiol) [59].

In one experimental study on alcohol alone and breast cancer, 20 female ICR mice were given 10–15 % ethanol solution as the *sole* drinking fluid for 25 months, with ad libitum solid diet [60]. Approximately, 45 % of mice developed either papillary or medullary adenocarcinoma of glandular epithelial origin. However, the relevance of this model for human alcohol consumption is questionable. Taking into account the average life span of ICR mouse, which is 2–2.5 years, these animals were given alcohol solution as the only drink available for about as much as 85 % of their life, at a rate equivalent to nonstop binge drinking, a situation that is neither physiological nor normal for humans.

Another study used transgenic mice that overexpress the HER2 protein (encoded by the proto-oncogene HER2/neu) in the mammary epithelium, resulting in the development of estrogen receptor alpha (ERα)-negative mammary tumors, similar to those of patients with HER2+ breast cancer [61]. Non-ovariectomized (NOVX) and ovariectomized (OVX) mice were exposed to 0, 5, and 20 % ethanol in the drinking water at 9 weeks of age till the endpoint (week 52), when serum was collected to determine estrogen levels. Tumor incidence in the 5 and 20 % alcohol-consuming NOVX mice was 53.33 and 66.67 %, respectively, compared to 40 % in the control mice; however, tumor incidence reached statistical significance only in mice consuming 20 % alcohol. Increase in tumor incidence was associated with increased systemic estrogen levels, increased expression of aromatase, and increased expression of ER-α in the tumors of 20 % alcohol-consuming mice. Additionally, ovariectomy blocked the effects of 20 % alcohol on tumor development (Fig. 2.1) despite the increase in estrogen levels due to alcohol. The authors concluded that "alcohol promotes mammary tumor development only in the presence of normal systemic estrogen levels, which the OVX animals lack," and "alcohol consumption

promotes HER2 breast cancer development via the estrogen signaling pathway." While 20 % alcohol consumption increased estrogen levels in OVX mice, the estrogen levels were still significantly lower than those of NOVX control mice. Also, OVX mice failed to develop tumors in numbers comparable to NOVX mice, which led the authors to state "estrogen may be important for the tumor model in general and that failure to see tumor promotion with alcohol is a secondary effect."

The results of this study highlight the importance of assessing the HER2 status in addition to that of ER and PR. To translate these results to humans, women who take estrogen-containing HRT could have an increase in breast cancer risk due to the combined effects of HRT and alcohol. However, the epidemiological study (#26 above) that took into account the HER2 status found that in premenopausal women with ER+/PR+/HER2+ tumors, the risk of developing breast cancer for those drinking 5 to ≤23 g/day was increased by 61 % and for ER−/PR−/HER2+ by 92 % for women drinking the same amount. It is apparent that the results of this epidemiological study do not dovetail in a straightforward manner with the mouse study, suggesting that the relationships between these variables are more complex.

The use of HRT that contains estrogen adds to the complexity of the interaction between various risk factors. For example, the Women's Health Initiative (WHI) reported that women who received ≥5 years of continuous treatment with estrogen and progestin have increased risk of breast cancer [62]. Similar results were reported by studies #12, #13, and #15 above, but not by #4. In the same WHI study, postmenopausal women with prior hysterectomy who received estrogen alone showed a statistically significant decrease in breast cancer risk [63]. In addition, women in the French observational E3N study who received estrogen alone or estrogens combined with micronized progesterone showed no increase in breast cancer risk; however, those who received estrogens and androgenic progestins, or who were on HRT for long time, were at increased risk [64]. In a Finnish study [65], postmenopausal women using estradiol (E2)-progestogen therapy showed no increase in breast cancer incidence within the first 3 years of use.

Since supraphysiological estrogen doses caused mammary adenocarcinomas in rats [66], and alcohol consumption increased plasma estrogen levels (not to a supraphysiological level) in human female volunteers [67], it was postulated that alcohol use should be more strongly associated with ER+ than ER− tumors. However, epidemiological studies that assessed the risk of alcohol consumption based on tumor status were contradictory. For example, while some epidemiological studies showed a modest increase in ER+ tumors with the consumption of 15–30 g/day, there was no association with consumption of >30 g/day (see study #8 above). The link between alcohol use and ER+ or PR+ status was not consistent across different studies (see discussion under #13). For instance, studies showed statistically nonsignificant associations with either or both ER+, PR+ for women consuming ≥10 g/day (study #2). Consumption of 30 g/day was associated with a modest increase in risk of ER+/PR+ tumors, but there was no increase in risk for ER+/PR− tumors (study #12). Other studies showed that 10 g/day of alcohol increased the risk in either ER+ or ER− (study #13), or mostly in ER−/PR− (study #14). Study #17 showed that consumption of 10–20 g/day was associated with 7 and

28 % increase in risk for ER+/PR+ and ER−/PR− tumors, respectively. Finally, meta-analysis of 4 prospective and 16 case–control studies [68] showed that an increase in alcohol consumption of 10 g per day was associated with increased risks for ER+/PR+ (11 %) and ER+/PR− (15 %). The authors concluded that the observed positive associations with alcohol for ER+/PR+ and ER+/PR− tumors cannot be explained by estrogen-dependent pathway only.

In addition, estrogen status is influenced by numerous exogenous factors. For instance, persistent exposure of mammary gland stem and progenitor cells to different environmental factors such as xenoestrogens (bisphenol A, phthalates, ethinyl estradiol, phytoestrogens) alters their epigenetic reprogramming during epithelial differentiation [69]. This is mediated, in part, through ERα nuclear receptors which activate or silence the transcription of target genes [70]. Interactions between ERα and various enzymes involved in histone modifications (histone acetyltransferases, histone deacetylases, histone methyltransferases, histone demethylases), co-activators, and co-repressors have introduced another layer of complexity in the epigenetic regulation of breast carcinogenesis [71]. Furthermore, women who were exposed in utero to diethylstilbestrol (DES), a synthetic estrogen, are at greater risk of developing breast cancer in their 40s (1.8–2.5-fold increased risk) and in their 50s (threefold increased risk) [72]. These environmental and epigenetic factors involving estrogen need to be taken into consideration in epidemiological studies.

Estrogen levels are intertwined with obesity to influence breast cancer risk. While some studies showed no effect of BMI on risk for breast cancer (e.g., study #20 above), dysregulation of sex hormones, hyperinsulinemia, and inflammatory cytokines in obese women are factors that could influence the risk for breast cancer. Obesity is significantly associated with low plasma levels of sex-hormone-binding globulin (SHBG), which increases the bioavailability of estrogens and androgens [73]. Thus, many established risk factors for breast cancer may function through an endocrine mechanism.

2.4.2.2 Alcohol Metabolism

The liver is the major organ for metabolizing ethanol mainly by oxidative pathway which involves cytosolic alcohol dehydrogenase (ADH), of which multiple isoenzymes exist—e.g., in humans, class I ADH is composed of three genes (ADH1A, ADH1B, and ADH1C)—to produce acetaldehyde, a highly reactive molecule. ADH acts on a wide range of substrates including retinol. The cytochrome P450 isozymes, mainly CYP2E1, predominantly present in the endoplasmic reticulum, also contribute to ethanol oxidation to acetaldehyde in the liver, particularly at higher alcohol concentrations. CYP2E1-dependent ethanol oxidation may occur in other tissues where ADH activity is low. CYP2E1 also produces highly reactive oxygen species (ROS), including hydroxyethyl, superoxide anion, and hydroxyl radicals. Acetaldehyde, produced by ethanol oxidation through any of these mechanisms, is rapidly metabolized mainly by mitochondrial aldehyde dehydrogenase (ALDH2) to form acetate and NADH and to a much lesser extent by ALDH1 in the cytosol.

Mitochondrial NADH is oxidized by the mitochondrial electron transport chain. Chronic alcohol consumption renders mitochondrial oxidative phosphorylation inefficient by interfering with the main respiratory complexes (Complex I, III, IV, and V) of the electron transport system encoded on mitochondrial DNA (mtDNA), resulting in the formation of the superoxide anion. In breast cancer, like in other cancers, mitochondrial function is severely impaired [74]. One early event in breast carcinogenesis can be mutations in mtDNA that destabilize the oxidative phosphorylation system (OXPHOS) which can result in a shift in energy metabolism toward enhanced aerobic glycolysis. Alcohol metabolism could influence breast carcinogenesis by generating acetaldehyde and ROS and by interfering with retinol metabolism.

Acetaldehyde is suspected in playing a role in breast carcinogenesis. Since blood acetaldehyde levels are very low or undetectable after alcohol consumption in humans [75], the human breast would not be exposed to significant levels of exogenous acetaldehyde. Thus, ADH activity and in situ generation of acetaldehyde in the human breast tissue after ethanol consumption is of potential significance. In mammary tissue of rats, cytosolic ADH and ALDH1 activities were 5.8 and 8.3 % of that in the liver of the same animals, respectively [76]. Similarly, mitochondrial ALDH2 activity in breast tissue was 7.1 % of that in the liver. In humans, studies on normal and neoplastic breast tissue showed that class I, but not class IV, ADH is expressed in human mammary epithelium, which can support ADH-mediated oxidation of ethanol; however, the expression of class I ADH is dramatically reduced or abrogated in invasive breast cancers [77]. The authors opined that this "virtual abrogation of expression of class I ADH in invasive breast cancer suggests that the enzyme has some 'tumor suppressor' function in the mammary epithelium." However, whether the reduction in class I ADH activity was causally related to tumor formation or was merely a bystander effect was not considered. To investigate acetaldehyde formation by the cytosolic pathway and the microsomal fraction in the mammary tissue, Sprague–Dawley female rats were injected intraperitoneally with 0.8 mL ethanol/kg/day for four consecutive days [78]. Mammary microsomal metabolism of alcohol to acetaldehyde by CYP2E1 was not induced after ethanol (or acetone) treatment, despite reports that CYP2E1 is expressed in normal and cancerous breast tissue [79]. In contrast, the cytosolic fraction of alcohol treated animals showed higher concentrations of acetaldehyde.

In humans, the interaction between alcohol consumption and ADH2 polymorphism with respect to breast cancer risk was reported in 278 German women with invasive breast cancer [80]. The authors stated that breast cancer risk associated with alcohol consumption may vary according to ADH2 polymorphism, probably due to differences in alcohol metabolism.

Variations in ADH and ALDH activities were reported to influence the risk of breast cancer. For example, while the *ADH1B* genotype [81] was not associated with breast cancer risk in a German population, a role for the *ADH1C* genotype has been suggested. This genotype, which is expressed mainly in the liver but also in breast tissue, has three polymorphic genes: *ADH1C1*1* and *ADH1C1*2* genotypes which result in enzymes with fast and intermediate turnover rates and which increase

the risk of breast cancer in Chinese drinkers, compared to *ADH1C2*2* which results in an enzyme with a slow rate of metabolism [82]. Similar results were obtained in a Long Island Breast Cancer Study which genotyped 1,047 breast cancer cases. Consumption of 15–30 g/day was associated with OR of 2.0, 1.5, and 1.3 in *ADH1C1*1*, *ADH1C1*2*, and *ADH1C2*2* genotypes, respectively [83]. Ironically, another study in Caucasian postmenopausal women found an association between risk of breast cancer and the slow metabolizing variant, which led the authors to conclude that "ethanol rather than acetaldehyde is related to breast cancer risk" [84]. However, two studies found no association between breast cancer risk and functional allelic variants of the *ADH1B* and *1C* genes [85] and *ADH1B* and *ALDH2*. The authors concluded that "our findings do not support the hypothesis that acetaldehyde is the main contributor to the carcinogenesis of alcohol-induced breast cancer" [86].

Acetaldehyde and NADH produced by alcohol metabolism can be substrates for xanthine oxidoreductase (XOR), which is inducible by alcohol and produces ROS, especially superoxide anion [87]. To add to the complexity, XOR also metabolizes (activates) nitrofurans and nitroimidazoles, chemicals that are used in veterinary medicine and by beekeepers in honey-producing hives. Therefore, residues of these compounds could exist in animal-derived foods and honey and might be involved in the associated mammary carcinogenic effects [88].

To explain acetaldehyde's role in carcinogenesis, scientists proposed a model in which acetaldehyde reacts with DNA to generate DNA lesions that form interstrand cross-links (ICLs). Cells are protected against replication blocking DNA lesions and ICLs through the Fanconi anemia-BRCA (FANC-BRCA) DNA-damage response network. Mutations in two major susceptibility genes, *BRCA1 and BRCA2*, which are involved in the maintenance of genomic integrity and DNA repair, were identified as major risk factors for breast cancer [89, 90]. The role of high levels of acetaldehyde in activating the FANC-BRCA network was discussed elsewhere [91, 92]. Furthermore, polymorphisms in the DNA repair gene *XRCC1* was associated with increased breast cancer risk in African-American women [93].

The role of acetaldehyde in breast carcinogenesis has not been evident in epidemiological studies. For example, study #19 above did not find a significant association between breast cancer risk and alcohol consumption in Asian American women, almost half of whom are deficient in ALDH2, the mitochondrial enzyme that metabolizes acetaldehyde. Furthermore, study #20 reported that the increase in risk for breast cancer in Japanese population was not modified by alcohol-induced facial flushing, which means the risk was not modified in women who have the defective ALDH2*2.

To examine alcohol effects on oxidative stress in the mammary tissue, female Sprague–Dawley rats were fed alcohol for 28 days [78]. An increase in hydroperoxide, but not the lipid peroxidation product malondialdehyde (MDA) concentration, and a significant reduction in glutathione in mammary tissue were observed. A study by Li and colleagues comparing breast cancer patients with cancer-free women reported that the levels of hydroxyl radical-DNA adducts and MDA-DNA adducts were ninefold higher in patient's normal breast tissue adjacent to tumor tissue than

in breast tissue from cancer-free controls [94]. These reports highlight the potential that oxidative stress may lead to DNA damage in cancer patients that is not evident in healthy women.

Class I ADH has the potential to catalyze the oxidation of retinol (vitamin A) to retinal [95], the first step in the biosynthesis of retinoic acid (RA), the principal mediator for maintaining epithelia in a differentiated state. Chronic and excessive alcohol intake interferes with retinoid metabolism and results in reduced RA. Alcohol acts as a competitive inhibitor of oxidation of vitamin A to RA (which involves ADH and ALDH) and induces CYP2E1, which can enhance catabolism of vitamin A and RA. The biological activity of RA is primarily mediated by nuclear retinoid receptors which are involved in the antitumor activity of retinoids. Studies indicate crosstalks between classic retinoids and various intracellular pathways controlling the growth, survival, and invasive/metastatic behavior of breast cancer cells [96]. Impaired RA homeostasis interferes with signaling (e.g., downregulates retinoid target gene expression) and with "crosstalk" with the mitogen-activated protein kinase signaling pathway (MAPK), including Jun N-terminal kinase and p38 kinase [97]. These observations could have implications for breast cancer prevention. However, better understanding of the alcohol–retinoid interaction and the molecular mechanisms involved is needed before it would be justified to pursue retinoids in the prevention of breast cancer. Nonetheless, retinoids could be potential components of innovative and rational therapeutic combinations for breast cancer. Yet, it is important to evaluate the responsiveness of ER+ tumors to retinoids and whether HER2 expression always plays a negative role in modulating retinoid sensitivity of HER2+/ER+ mammary tumors, as suggested by some studies [98].

2.4.2.3 Folate Metabolism/Epigenetic Factors

Mutations in oncogenes and tumor suppressor genes result in specific gene expression profiles that are involved in the regulation of cellular homeostasis, including cell proliferation and DNA repair and survival. However, differentiation of mammary stem cells to primitive progenitor cells is under epigenetic control. Epigenetic mechanisms, which result in changes in gene expression patterns without altering DNA sequence, partake in mammary glands developmental phases from in utero to menopause, as well as in breast carcinogenesis. One of these epigenetic mechanisms is DNA methylation [99].

DNA methylation involves the transfer of a methyl group from S-adenosylmethionine (SAM)—by DNA methyltransferases (DNMTs)—onto the 5′-position of the cytosine residue found in cytosine guanosine dinucleotide pairs (CpG). SAM is generated from methionine. After the methyl transfer reaction, SAM forms S-adenosylhomocysteine, which is then broken down to homocysteine. The latter can be remethylated to form methionine, by transferring a methyl group either from N5-methyltetrahydrofolate (THF) by methionine synthase or from betaine by betaine-homocysteine methyl transferase. Hypermethylation of CpG groups renders affected loci inaccessible to transcription factors resulting in transcriptional

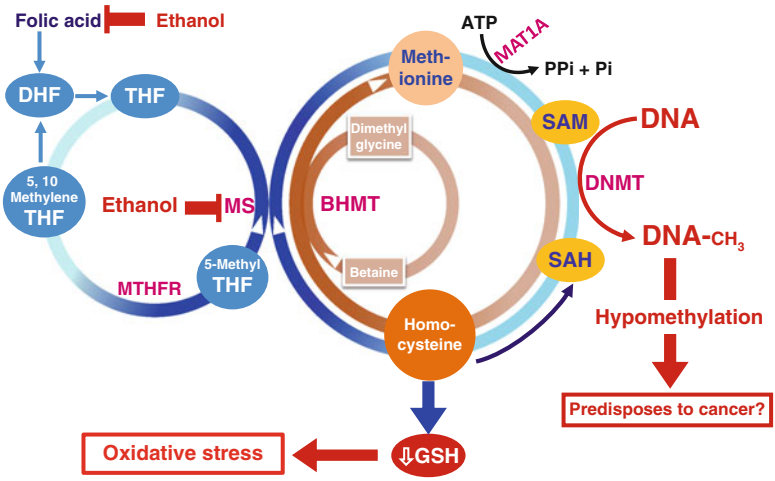

Fig. 2.2 Alcohol's effects on homocysteine/methionine metabolism and DNA methylation. *MTHFR* methylene tetrahydrofolate reductase, *MAT* methionine adenosyltransferase, *HCC* hepatocellular carcinoma, *BHMT* betaine homocysteine methyltransferases, *GSH* glutathione, *ATP* adenosine triphosphate, *Pi* inorganic phosphate

silencing. Importantly, CpG methylation in promoter regions of tumor-suppressor genes (e.g., *BRCA1*) leads to the inactivation of these cancer-preventing proteins. Similarly, hypermethylation of numerous genes, whose biological function include hormone regulation, DNA repair, cell cycle regulation, tissue remodeling, apoptosis, cell adhesion and invasion, cell growth inhibition, and angiogenesis, has been identified in breast tumors [100]. Furthermore, DNA hypermethylation results in aberrant regulation of the Wnt pathway in breast cancer [101]; BRCA1 expression is suppressed by a combination of gene mutation and DNA hypermethylation [102]. One epidemiological study of the interactions between alcohol consumption and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers reported no significant interaction with *BRCA1* mutations but a greater risk of alcohol-associated breast cancer in women with *BRCA2* mutations [103]. In fact, the same investigators reported an inverse association between breast cancer and current alcohol consumption in women with a *BRCA1* mutation [104]. Another study reported no association between alcohol intake and breast cancer risk for women with *BRCA1* or *BRCA2* mutations and suggested a possible reduction in risk in *BRCA2* mutation carriers with "modest" alcohol intake [105].

DNA hypomethylation can also contribute to breast carcinogenesis [106]. For example, promoter hypomethylation could reactivate the expression of certain proto-oncogenes (such as *synuclein γ*) which are associated with tumor metastasis [107].

Chronic heavy alcohol consumption leads to substantial DNA hypomethylation as a result of significant reduction in tissue SAM (Fig. 2.2). Additionally, alcohol perturbs the folate cycle that is involved in the methionine metabolic pathway, which is integral to supplying the methyl groups necessary for DNA methylation. Folate is an important nutrient required for DNA synthesis, and at least 30 different enzymes are involved in the complex folate cycle including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR). Defects or polymorphic variations in the folate metabolic pathway may influence cancer susceptibility. However, a study on 1,063 women with breast cancer found no association between MTHFR genotype and risk for breast cancer, and there was no evidence of an interaction of genotype and alcohol consumption in premenopausal women. However, in postmenopausal women, there was an increase in breast cancer risk in those who were homozygote *TT* for *MTHFR C677T* and drank >1.9 drinks/day [108].

Chronic heavy alcohol consumption can cause relative folate deficiency due to the negative effects of alcohol on folate metabolism, including malabsorption, increased excretion, or enzymatic suppression. Based on the above interactions between folate and alcohol, it would be expected that high folate intake should ameliorate the association between alcohol intake and risk for breast cancer that is caused by this mechanism. Examination of epidemiological studies revealed inconsistencies in the findings. For example, studies discussed under #25 and #26 showed that folate intake attenuated the alcohol-associated risk for breast cancer, so did another epidemiological study in Anglo-Australian [109] women aged 40–69 years; whereas studies discussed under #20 and #23 showed no association. Other studies reported that high intake of folic acid increased the risk of breast cancer in postmenopausal women enrolled in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial [110] cohort in the USA. Additionally, a case–control study in 570 Thai women concluded that genetic polymorphisms in folate and alcohol metabolic pathways may contribute to the etiology of breast cancer among Thai women [111]. In conclusion, the impact of folate supplementation on the risk for alcohol-induced breast cancer probably is affected by a wide range of other factors that are not well understood.

2.5 Concluding Remarks

Breast cancer is the most diagnosed cancer in women worldwide; it is one of the primary causes of death among women globally. Women, particularly if they have known genetic susceptibilities, should consult with their physician about risk factors involved in breast cancer. Overconsumption of alcohol is a risk factor for many diseases and one that women with or without a high risk for breast cancer are well advised to avoid,

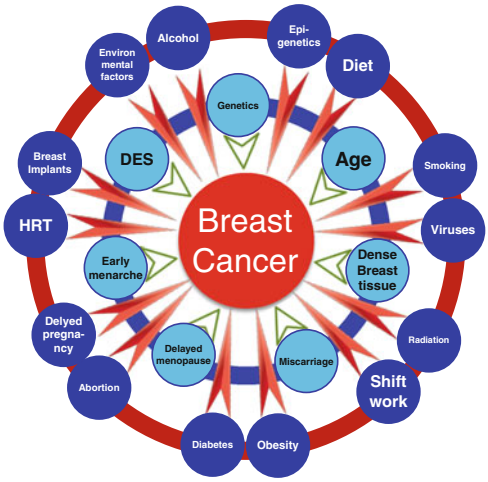

Fig. 2.3 Multiple risk factors associated with breast cancer

The question whether a woman should not drink at all in order to reduce the risk of breast cancer or may drink moderately without undue risk is not settled. The reasons are threefold: (1) there are at least 20 recognized risk factors that can affect the onset and outcome of breast cancer (Fig. 2.3) and the overall risk depends on the interactions of all these factors, including those that have not yet been identified; (2) epidemiological studies resulted in contradictory associations between the amount of alcohol consumed and risk for breast cancer; and (3) discrepancies exist between epidemiological and molecular studies.

2.5.1 Multiple Risk Factors

Numerous risk factors are involved in breast carcinogenesis; only a few of them will be discussed below to illustrate the complex interactions between these risk factors. Some of the risk factors associated with breast cancer are genetic and beyond the control of a woman. Mutations in *BRCA1 and BRCA2*, were identified as major risk factors for breast cancer [89, 90]. However, the incomplete penetrance of these mutations suggests that other factors, environmental and hormonal, may modify that risk, for example, a study in which monozygotic (MZ) twins who carried identical *BRCA1* gene mutation resulted in discordant phenotypes; one suffered from breast cancer twice in 27 years while her MZ twin remained healthy [112].

The majority of breast cancers are not hereditary. Most late-onset breast cancer occurs in the absence of a first-degree family history of breast cancer. Genome-wide association studies have identified genetic susceptibility variants of medium-penetrance [113–116] (which confer risk of 2–3-fold per allele) and modest penetrance (which increase the risk 1.1–1.3-fold per allele). However, these variants could explain only

20–25 % of familial breast cancer risk [117]. These findings led to the hypothesis that susceptibility to breast cancer is polygenic, i.e., conferred by a large number of loci, each with limited contribution to breast cancer risk [118].

Age is another important nonmodifiable risk factor. Invasive breast cancer or its precursor lesion DCIS occurs at an exponential rate until about age 50 (menopause) followed by a slower rate of increase [119], supporting the notion that breast cancer biology is age-dependent. Early-onset breast cancer, therefore, could largely represent inherited mutations (*BRCA1*, *BRCA2*, *TP53*, *ATM*, *or PTEN*) or early life transforming events that affect the immature mammary cells [120]. In contrast, late-onset breast cancer could be due to an early mutagenic initiating event, which is then subjected to later life exposure to endogenous or exogenous promoting agent(s) and further compounded by age-related impairments in macromolecular repair, immune surveillance, or xenobiotic detoxification. This could explain the increased risk from HRT.

Risk factors are greatly influenced by epigenetic mechanisms that change gene expression patterns for cell differentiation, proliferation, and apoptosis. These epigenetic mechanisms include DNA methylation, histone modifications, and the effects of noncoding RNAs such as microRNAs (miRNA) [99]. Epigenetic changes that influence the transciptomes, proteomes, and metabolomes and ultimately breast cancer evolution, are brought about by numerous endogenous (e.g., hormonal, microbiota, aging, inflammation, inherited diseases such as type 1 diabetes) and exogenous (diet, smoking, infection, alcohol, obesity, radiation, shift work, circadian rhythm disturbances) etiological factors that result in the vast heterogeneity of breast cancers. In fact, a study on the clinically relevant subtypes, luminal A and basal-like breast cancer revealed that distinct molecular mechanisms might have been preprogrammed at an early stage of the disease and that these breast tumor subtypes represent biologically distinct disease entities that may require different therapeutic strategies [121].

The interactions between genes and the environment are crucial. Environmental factors add another layer of complexity by their interactions with the susceptibility genes for breast cancer and for metabolic diseases. For example, in a twin study in Finland [122], the probability that a co-twin would develop breast cancer (given that one twin already had breast cancer) was 10 % for monozygotic and 8 % for dizygotic twins, suggesting that combined environmental effects are dominant in the development of breast cancer. In addition, in a subset of the Million Women Study (study #11 above), the strongest suggestion of a gene–environment interaction was between the high-risk common variant CASP8-rs1045485 and alcohol consumption; the per-allele relative risk of breast cancer for CASP8-rs1045485 was not increased by consuming <1 drink/day, but was increased by 23 % (nonsignificant) in women who reported consuming one or more alcoholic drinks/day [123]. Another study found no interaction between the breast cancer susceptibility locus CASP8-rs17468277 and consuming <20 g/day of alcohol; however risk was increased by 45 % in those who drank ≥20 g/day [124]. Therefore, epidemiological studies should focus on gene–environment interactions rather than singling out individual risk factors as if they operate in isolation.

2.5.2 *Epidemiological Studies*

While epidemiological studies, when conducted properly, can be effective tools to identify the root of health problems and disease outbreaks in a community, epidemiological studies that deal with alcohol consumption and its consequences should be interpreted with considerable caution. Such studies can only highlight associations and cannot determine cause-and-effect relationships. Considering the wide range of variations in the size of studies, measurement errors in input and outcome variables, and individual variations in genetic background and life style factors, many of which were not taken into account, it is not surprising that the results have been contradictory. The epidemiological studies on alcohol and breast cancer discussed above point to the following important points as possible sources of the variability in outcomes:

1. All studies use self-report to determine the amount and type of alcoholic beverage consumed. Most of the studies acknowledged "recall bias" that makes the alcohol consumption variable notoriously inaccurate, and drinking pattern could change during the period under consideration. In fact, a recent study on moderate alcohol intake and cancer stated that the apparent increased risk of cancer among light-moderate drinkers may be "substantially due to underreporting of intake" [125]. There is now strong evidence that recall over prolonged periods tends to grossly underestimate actual drinking frequency. This was highlighted dramatically in a recent study by Stockwell et al. [126] who compared Quantity-Frequency (QF) recall with beverage-specific "yesterday" (BSY) consumption reports and with alcoholic beverage sales reports in a Canadian population to demonstrate that underreporting of alcohol consumption was considerable (2–3-fold) and varied by age and consumption level. Particularly relevant is the finding that moderate drinkers underreport their drinking much more than more frequent drinkers. In many of the epidemiological studies summarized above, an underreporting by a factor of 2–3 would increase the alcohol consumption associated with increased risk of breast cancer into the recognized risky drinking category. Other studies used as input of alcohol consumption "ever" vs. "never" without even quantification, and others use inaccurate information to characterize drink size (e.g., study #1 characterizes a drink of a liquor as one ounce containing 15.1 g of alcohol). The definition of a standard drink according to the National Institute on Alcohol Abuse and Alcoholism [127] and the Dietary Guidelines for Americans is: 12 oz of beer (5 % alcohol), 5 oz of wine (12 % alcohol), and 1.5 oz of distilled spirit (40 % alcohol). In addition, the Dietary Guidelines for Americans define moderate drinking as consuming no more than one drink/day for a woman, and no more than 2 drinks/day for a man. Although some studies used FFQ and claim it as valid, lack of consumption ascertainment can result in confusing results. For example, in study #4 above, consumption of 1 or 2 drinks/day increased breast cancer risk by 40 %, whereas consumption of two or more drinks/day resulted in no increase in risk. A 42 % decrease in breast cancer risk was associated with drinking <1.5 drink/day (study #5),

whereas drinking 3–6 drinks/week was associated with a 15 % increase in risk (study #2). In fact, a meta-analysis reported by Longnecker (#3) stated "the modest size of the association and variation in results across studies leave the causal role of alcohol in question."

2. Patterns of consumption are rarely analyzed. The majority of studies use average weekly consumption. Drinking seven drinks on a Saturday night and nothing the rest of the week is not, health-wise, the same as having one drink every day of the week.
3. Breast cancer develops over a relatively long period of time, often more than 20 years [128], and thus, correlations between alcohol consumption and breast cancer cannot be determined in epidemiological studies with windows of alcohol exposure that captures current or recent alcohol intake, after clinical diagnosis; for example, studies # 4, 6, 14, and 21 assessed recent alcohol consumption, consumption 2 years prior to diagnosis, past-year drinking, or consumption 3 months after diagnosis, respectively.
4. Despite the fact that age per se is a risk factor for breast cancer and during aging other environmental factors may compound or modify the risk, most epidemiological studies stratify data based on pre- or postmenopausal status alone. Thus, including women (ages 20–74, 25–85, 40–75, 23–74, and 20–87 in studies # 4, 5, 7,16, and 21, respectively)—who have various degrees of exposure to environmental and lifestyle factors that influence the genesis of breast cancer—without stratification could be confounding. Similarly, studies that took into account other risk factors such as smoking, BMI, use of HRT, etc., resulted in contradictory findings (see the above cited studies).
5. Results of the association between breast cancer risk and alcohol consumption based on ER, PR, and HER2 status are very contradictory and vary between different studies. Therefore, correlations with HRT use cannot be deciphered, based on currently available data.
6. Rarely does an epidemiological study differentiate between specific subtypes of breast cancer (there are about 20 different types) and risk of alcohol consumption. These different subtypes, as described above, are heterogeneous, have distinct molecular mechanisms, are associated with unique risk factors, and might have been preprogrammed at an early stage of the disease.
7. In a recent article, Ogino and colleagues [129] advocated the use of "Molecular Pathological Epidemiology" (MPE) to understand the interplay between etiological factors, cellular molecular characteristics, and disease evolution in multifactorial diseases such as cancer. While conventional molecular epidemiology generally considers a disease as a single entity, MPE integrates analyses of population studies together with the macroenvironment and molecular and microenvironment. This approach will allow scientists to investigate the relationships between potential etiological agent and disease subtypes based on molecular signatures. This concept is similar to systems biology approach and, according to the authors, "enables us to link potential etiological factors to specific molecular pathology, and gain novel pathogenic insights on causality." This is a very important concept that needs to be applied to alcohol and breast cancer.

2.5.3 Discrepancies Between Epidemiological and Molecular Studies

A major challenge in understanding the epidemiological findings is to elucidate the biological basis underlying the association. Several molecular mechanisms have been postulated, including formation of acetaldehyde and ROS, epigenetic effect through the folate cycle, and estrogen formation. However, there seems to be discord between molecular and epidemiological studies.

(a) Acetaldehyde formation

Molecular studies have demonstrated the presence of alcohol-metabolizing enzymes ADH, ALDH1, and ALDH2 in breast tissue. Since acetaldehyde can form adducts with DNA and result in DNA lesions, it would seem logical that genes that encode for fast metabolizing ADH or defective ALDH2*2 enzymes that result in acetaldehyde accumulation would increase the risk for breast cancer. While some studies such as the Long Island Breast Cancer Study showed an increased risk in fast metabolizers; other epidemiological studies in Asian American women and in Japan (# 19, 20) showed that breast cancer risk was not significantly associated with alcohol drinking and that facial flushing associated with the defective ALDH2*2 did not modify risk for breast cancer.

(b) Folate metabolism

Alcohol's effects on folate absorption and metabolism are well documented. Consequently, it is expected that levels of folate consumption can have a protective effect on breast cancer among women who consume alcohol. While some studies concluded that folate has protective effect on breast cancer in alcohol-consuming women, the Women's Health Initiative-Observational Study found no evidence for folate attenuating alcohol's effect on breast cancer risk. Similarly, the American Cancer Society Cancer Prevention Study II Nutrition Cohort reported no association between risk of breast cancer and dietary folate, total folate, or methionine intake, and there was no evidence of an interaction between dietary folate or total folate and alcohol. For more discussion, the reader is referred to Sect. 2.4.2.3 under molecular mechanisms.

(c) Estrogen metabolism

See discussion above about estrogen metabolism.

In summary, despite all the effort, there is no solid evidence associating moderate alcohol consumption with an increased incidence of breast cancer. A woman and her physician should weigh the risks and benefits of moderate alcohol consumption, which could be a part of a healthy life style. This is especially important in light of the fact that moderate alcohol consumption has been associated with potential health benefits, including decreased risk of coronary artery disease and overall mortality, protection against congestive heart failure, decreased risk of ischemic stroke, and protection against type 2 diabetes and rheumatoid arthritis.

Confronting the huge global breast cancer issue should be based on sound science. The lack of unambiguous reliable information about alcohol and breast cancer has opened the door to various unsubstantiated opinions on the subject and

to errant hypotheses about causes and prevention. That caused a great deal of confusion among women about the subject. Thus, confirming association between breast cancer and alcohol consumption, if any, should be a high priority. Systematic studies should be based on a large cohort; ascertain alcohol consumption by reliable and validated methods, preferably over prolonged periods, take into account the interaction with the multitude of other risk factors; and incorporate detailed biological information, including breast cancer subtypes. Public health policies must be rooted in impeccable science.

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