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Full Length Research Paper

Preventing hereditary cancers caused by opportunistic carcinogens

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Abstract

Inheritance of a BRCA1/2 defect predicts such high breast cancer risks that prevention has not been widely studied. Hereditary BRCA1/2 gene mutations can cause cancer by impairing protective responses to some carcinogens. Literature searches identified formaldehyde and acetaldehyde as contributors to mutation related carcinogenesis if detoxification pathways are overwhelmed. The work first determined whether the types of DNA damage associated with hereditary cancers resemble DNA damage caused by the two opportunistic carcinogens. Both carcinogens activate BRCA1/2 pathways; both carcinogens cause the same types of DNA damage found in BRCA1/2 related cancers; both carcinogens increase risks for leukemias theoretically and statistically associated with BRCA1/2 deficiencies. Rats given acetaldehyde have increased incidence of malignant mammary tumors, leukemias, lymphomas, and pancreatic tumors. In humans, breastfeeding transmits acetaldehyde to infants. At least seven studies link acetaldehyde to early onset breast cancer. Risks from these and other opportunistic carcinogens including radiation may be modified in BRCA mutation carriers. Compensating for the genetic deficit may prevent or delay some hereditary cancers.

Keywords: BRCA1, BRCA2, hereditary breast cancer, breast cancer, ovarian cancer, familial cancer, environmental cancer, inherited cancer, breast cancer genes.

INTRODUCTION

Understanding the tissue specificity of hereditary breast cancer

Although all cells inherit the same mutation, BRCA1 and BRCA2 mutations are widely believed to specifically target breast and ovary for cancer. This has been attributed to some special property of the inherited gene mutation. Previous work (Friedenson, 2010b) suggested that risks for hereditary cancers are stimulated because specific organs are exposed to carcinogens that can take advantage of the inherited gene deficit to cause cancer. Mutations in other genes also contribute to cancer risks, but identifying and then controlling exposure to opportunistic carcinogens that take advantage of known BRCA1/2 gene mutations is a first step in preventing or delaying some hereditary cancers.

In carriers of mutations in pathways dependent on BRCA1/2 genes, there is an increased incidence of cancers (Friedenson, 2010a) related to chronic organ specific infections that cause DNA damage (De Marzo et al., 2007; Machida et al., 2006; Sutcliffe et al., 2007). The first defenses against these chronic infection induced cancers are encoded by genes for immune responses, and for processing, detoxifying and metabolizing carcinogens. If the infection is not cleared BRCA1/2 mediated pathways serve as further defenses to repair certain types of DNA damage (Maul et al., 1998), especially damage caused by DNA cross links (Friedenson, 2010a, b). Inherited mutations can cripple these repairs and other BRCA1/2 protective functions. Constant cell death and replacement then occur in a mutagenic environment. Inherited deficiencies in BRCA1/2 pathways exaggerate

Abbreviations

ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; DSBreaks, double strand breaks; matrix metalloproteinase-9 MMP-9; MLL, Multi Lineage Leukemia; NPM, nucleophosmin

cancer risks from exposure to opportunistic carcinogens that overwhelm detoxification pathways.

Here, types of DNA damage associated with BRCA1/2 related hereditary cancers are shown to be the same types of DNA damage caused by two opportunistic carcinogens. The two carcinogens cause DNA cross links that depend on BRCA1/2 pathways for repair (Abraham et al., 2011; Lu et al., 2010; Theruvathu et al., 2005). Similar cross links are found in BRCA1/2 related cancers. Another criterion is that mutation carriers have higher risks for cancers known to be associated with particular types of carcinogens. Even though this evidence does not prove a direct cause and effect relationship, it does suggest that mutation carriers can reduce their cancer risks by limiting or compensating for carcinogenic damage that requires BRCA1/2 related pathways for repair.

Materials and Methods

Comparing DNA damage associated with pathway deficits to DNA damage caused by carcinogens

PubMed, Google and Google scholar indexes were searched for articles published since 1950. Search criteria for identifying opportunistic carcinogens were as follows: exposure that is common and widespread; a large amount of available information; carcinogenic damage that likely requires BRCA1/2 pathway repairs; a likely increase in risks for any cancer; and evidence for effects on stem cells. Formaldehyde and acetaldehyde were identified as opportunistic carcinogens with exposure being common through the diet or pollution. DNA damage found in retrieved studies of BRCA1/2 pathway deficits was compared to DNA damage reported for formaldehyde and acetaldehyde/alcohol. Then any cancers caused by these carcinogens in animals or humans were listed and compared to cancers linked to BRCA1/2 pathway deficits. Systematic searches were then conducted for relative risks for human cancers in potential mutation carriers caused by exposure to formaldehyde or acetaldehyde. Potential mutation carriers were defined as those having known risk factors for a pathogenic mutation in BRCA1/2 genes or within a pathway depending on BRCA1/2 genes (Friedenson, 2007).

Included risk data was used directly without further statistical calculation, adjustment, or combination. Included data was not corrected for low percentages of mutation carriers in the population, for survival, or for mortality causing a loss to follow-up. Studies of second primary cancers after breast cancer provided the most extensive and complete sources of statistical cancer risk data for BRCA1/2 heterozygotes. Given the high risk of BRCA1/2 mutation carriers and their involvement with medical oncology personnel, cancer survivors among patients with BRCA1/2 pathway mutations are often monitored for the development of a second primary cancer. This is done more often than observing genetically typed healthy individuals for the development of a first cancer. Relationships between alcohol consumption and cancer risks can only be studied in populations with substantial alcohol consumption (Seitz and Becker, 2007; Seitz and Stickel, 2007) and epidemiologic studies can easily be confounded by very high levels of acetaldehyde in some diets (Salaspuro, 2009). Epidemiologic studies could not be used if they contained very high percentages of non-drinkers or light drinkers (>=70% to 98%) among potential BRCA1/2 mutation carriers. Substantial differences in numbers of oophorectomies or women on hormone replacement therapy in drinkers vs. controls also excluded data.

RESULTS

Formaldehyde and acetaldehyde produce the same types of DNA lesions found in hereditary cancers associated with deficits in BRCA1/2 pathways

Table 1 compiles evidence showing that at least four types of genetic mutations found in cancers associated with BRCA pathway deficits are the same types of DNA mutations caused by formaldehyde and acetaldehyde. If the two carcinogens exceed the capacity of detoxification pathways (Figures 1 and 2), they can go on to cause genetic damage. Formaldehyde and acetaldehyde have the ability to chemically cross-link DNA strands and to form DNA protein cross links (Figure 1 and 2) (Ishikawa et al., 2007; Shaham et al., 1996; Zhang et al., 2010). These kinds of DNA lesions are widely accepted as critical for BRCA1/2 pathway associated hereditary carcinogenesis. Such cross links are not repaired or mis-repaired in homozygotes with deficits in BRCA1/2 pathways. Deficits in repairing such cross links may result in double strand breaks and may be associated with more frequent gene rearrangements (Ashworth, 2008; Friedenson, 2007).

Although p53 is mutated in many cancers, p53 gene mutations are characteristic of BRCA1/2 associated hereditary cancers and found in almost all (96%) ovarian cancers (Cancer Genome Atlas Research, 2011). p53 gene mutations are also caused by formaldehyde and acetaldehyde exposure. Table 1 shows that epigenetic modifications caused by the two carcinogens are consistent with a global reduction in methylation levels with aberrant- or hyper- methylation of tumor suppressor promoters.

The last row in Table 1 notes that loss of BRCA1/2 and exposure to acetaldehyde and probably formaldehyde are all associated with increased activity of metalloproteinases. Metalloproteinase enzymes are thought to facilitate invasion and metastasis (Table 1).

Table 1. The same types of genetic mutations in cancers associated with BRCA pathway deficits are caused by aldehyde carcinogens

DNA damage associated with formaldehyde acetaldehyde	Association of this type of DNA damage and BRCA pathway related cancers	Evidence that formaldehyde or acetaldehyde cause this type of DNA damage and that this damage is present in cancers associated with these carcinogens
DNA protein cross links or DNA-DNA cross links	Cells with truncated Brca2 are hypersensitive to DNA damage by interstrand cross-linkers. Even low doses trigger aberrant genetic exchange between non-homologous chromosomes leading to gross chromosomal rearrangement (Yu et al., 2000). Large chromosome rearrangements are visible in chromosome spreads in cancer prone hereditary diseases: Fanconi anemia, BRCA deficiency (Ashworth, 2008) and Ataxia telangiectasia. BRCA2 mutations are well known in hereditary breast cancers and are also frequent in esophageal cancer depending on geographic locations, showing environmental influence (Zhong et al., 2011). Repair of interstrand cross link involves both BRCA1/2 proteins and Fanconi proteins (Huen et al., 2010). Repairs mediated by BRCA pathways are absent in MLL (see Fig. 3). Brca1 contributes to damage repair and/or tolerance by promoting assembly of Rad51 (Bhattacharyya et al., 2000).	Both carcinogens cause DNA protein cross links as critical carcinogenic lesions that require repairs mediated by BRCA1/2 pathways (Cho et al., 2006; Fang and Vaca, 1997; Marietta et al., 2009; Nakano et al., 2009; Theruvathu et al., 2005). In *e coli* exposed to even low doses of formaldehyde, at least 50% of DNA protein cross links bind the two strands of DNA together (Wilkins and Macleod, 1976). In mice exposed to 6 ppm formaldehyde for 1 week, the Brca pathway is among the top ten most significantly enriched pathways (Andersen et al., 2010) Acetaldehyde causes a concentration-dependent increase in Fanconi D2 monoubiquitylation, which is dependent upon the proper functioning of BRCA1/2 (Fig. 3). Acetaldehyde also stimulates BRCA1 phosphorylation in a dose-dependent manner. Acetaldehyde increases the frequency of sister chromatid exchanges and aberrations in mammalian cell model studies. Fanconi anemia cells (with a defect in BRCA dependent pathways) are much more susceptible than normal cells(Obe and Anderson, 1987). Acetaldehyde is linked to esophageal cancer (Lachenmeier et al., 2009) and alcohol/acetaldehyde is linked to breast cancer in many studies. Formaldehyde is a proven cause of myeloid leukemia and BRCA1/2 pathways are linked to myeloid leukemia in Fig 3.
p53 gene mutation	Both in vitro and in vivo, BRCA1 physically associates with the p53 tumor suppressor and stimulates its transcriptional activity, serving as a p53 coactivator (Zhang et al., 1998). Mutations in p53 allow cells with mutations in both alleles of BRCA1 to survive, preventing embryonic lethality and facilitating cancers. Combined inactivation of BRCA2 and p53 mediates mammary tumorigenesis. Disruption of the p53 pathway is pivotal in BRCA2-associated breast cancer (Jonkers et al., 2001).	p53 gene mutation occurs in formaldehyde carcinogenesis. Workers with chronic formaldehyde exposure have increased risks for p53 mutations (Shaham et al., 2003). Acetaldehyde causes p53 gene mutations in esophageal cancer cells (Paget et al., 2008) with predominant G > A transitions and mutations at A:T base pairs (Noori and Hou, 2001).

Table 1 cont.

Methylation	Expression patterns of BRCA1, pRb, p16, PTEN and p53 strongly correlate with each other. Hypermethylation of the tumor suppressors pRb and p16 correlate with alcohol and tobacco use in upper aerodigestive tract cancer (Ishida et al., 2005). The BRCA1 promoter becomes hyper methylated in sporadic breast and ovarian cancers, suggesting a mechanism for inactivating the normal allele and other tumor suppressors in hereditary cancers (Cancer Genome Atlas Research, 2011). Epigenetic inactivation of BRCA1 is associated with pathology also prevalent in tumors from BRCA1 mutation carriers, including estrogen receptor negativity and medullary and mucinous tumors (Stefansson et al., 2011). Methylation of tumor suppressor genes increases in normal tissues in Fallopian tube carcinoma (a common origin for hereditary ovarian cancer) (Bijron et al., 2011). Aberrant methylation was observed for BRCA2 and other breast cancer related genes. In laryngeal squamous cell carcinoma samples, FANCA, BRCA1 and BRCA2 promoter regions had recurrent alterations in methylation levels (Szaumkessel et al., 2011). Hypermethylation of promoter regions from Fanconi-BRCA genes occurs in sporadic leukemia, albeit infrequently (Hess et al., 2008).	Formaldehyde and acetaldehyde alter regulation of DNA methyltransferases (Garro et al., 1991; Liu et al., 2011). Global hypomethylation and aberrant promoter methylation or hypermethylation of tumor suppressor genes are frequent in human cancers (Daniel et al., 2010). During 24 weeks formaldehyde exposure, genomic DNA methylation levels gradually decreased in a time-related manner. Rats fed ethanol have altered tissue specific methylation of some genes (Wani et al., 2011). DNA methylation of multiple genes, especially hypermethylation of the p14ARF tumor suppressor promoter, is common in oral squamous cell carcinoma and associates with tobacco and/or alcohol usage (Ishida et al., 2005) Ethanol causes site selective acetylation, methylation, and phosphorylation in histones.
Matrix metallopro-teinase-9 (MMP-9), NFKB, TNF alpha	Loss of BRCA2 promotes prostate cancer cell invasion through up-regulation of matrix metalloproteinase-9 (Moro et al., 2008) which associates with poor survival in breast cancer. Ras-transformed BRCA1-deficient mammary tumor cells appeared to secrete matrix metalloproteinases (Navaraj et al., 2009)	Acetaldehyde induces MMP-9 gene expression in liver cancer facilitating invasion (Gosepath et al., 2006). MMP-9 associates with NF-κB and TNF α elevation. In nasal epithelium, NF-kB and TNF α expression increased (MMP-9 not measured) (Gosepath et al., 2006).

BRCA1/2 deficits associate with myeloid leukemias

This section shows that myeloid leukemia associates with inherited deficiencies in BRCA1/2 pathways (Fig. 3) and the next section shows that myeloid leukemia also associates with the carcinogens formaldehyde and acetaldehyde. Table 2 shows multiple relationships between myeloid leukemias and deficits in pathways containing BRCA1/2 (Figure 3). Balanced translocations in myeloid leukemias involving the BRCA1 locus suggest some pathogenic mechanisms in leukemias are shared by hereditary breast cancers. It is now accepted that fusion genes not only are the hallmark of hematological malignancies and sarcomas, but also play an important role in epithelial cell carcinogenesis (Mao et al., 2011). All leukemias display aberrant distribution of cytosine methylation, which is most notably distributed in specific and distinct signatures in AML.

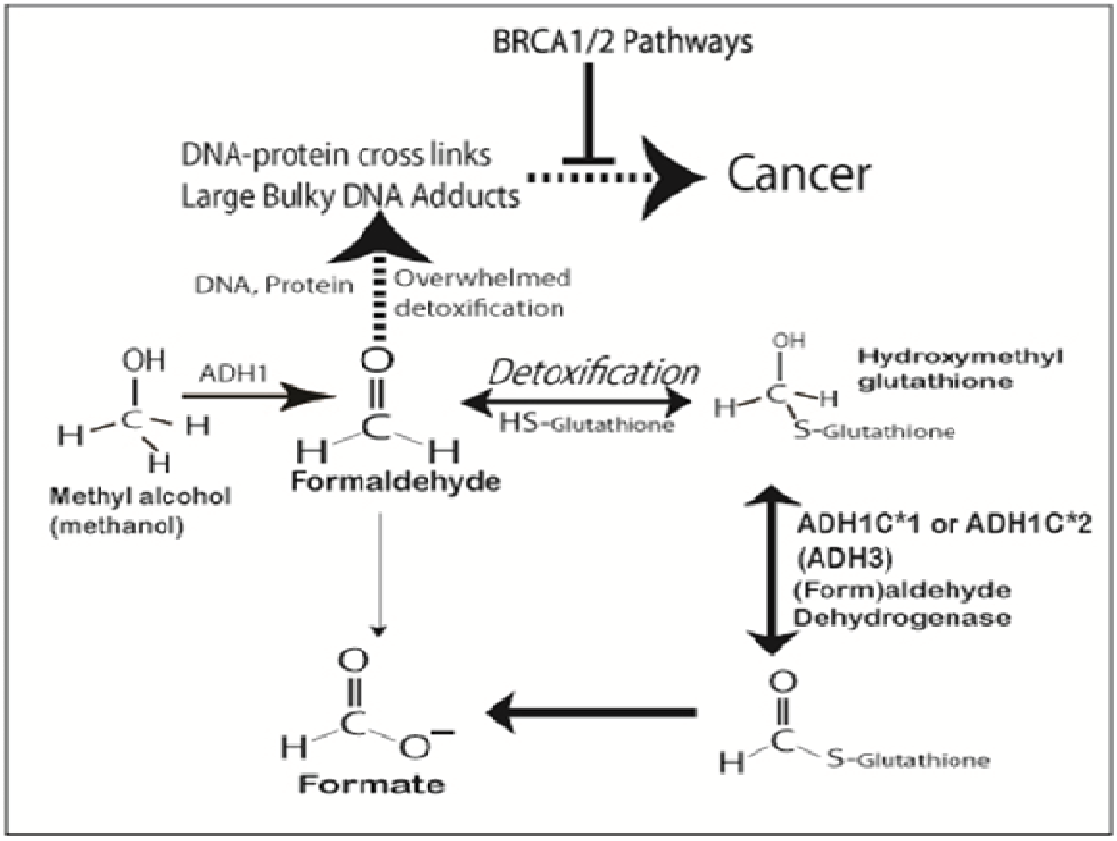

Figure 1. Carcinogenesis at high levels of formaldehyde competes with formaldehyde detoxification mechanisms. Formaldehyde that overwhelms detoxification mechanisms causes bulky DNA addition products, DNA cross links or hypermethylation of promoters encoding DNA pathway proteins. Detoxification of lower levels of formaldehyde occurs primarily by a pathway (thicker arrows) involving formaldehyde dehydrogenase. The pathway converts formaldehyde to formate which is then eliminated in the urine, broken down to CO2 and water or enters the single carbon pool. Alternate, less used pathways are indicated by thinner arrows. Formate can also generate CO2-radicals and can be metabolized to CO2 via catalase or oxidation of N-formyl tetrahydrofolate. Detoxification of formaldehyde does not involve BRCA1/2 but BRCA1/2 pathways inhibit carcinogenesis. Alternate, less used pathways are indicated by thinner arrows. Formate can also generate CO2 radicals and can be metabolized to CO2 via catalase or oxidation of N-formyl tetrahydrofolate. Detoxification of formaldehyde does not involve BRCA1/2 but BRCA1/2 pathways inhibit carcinogenesis.

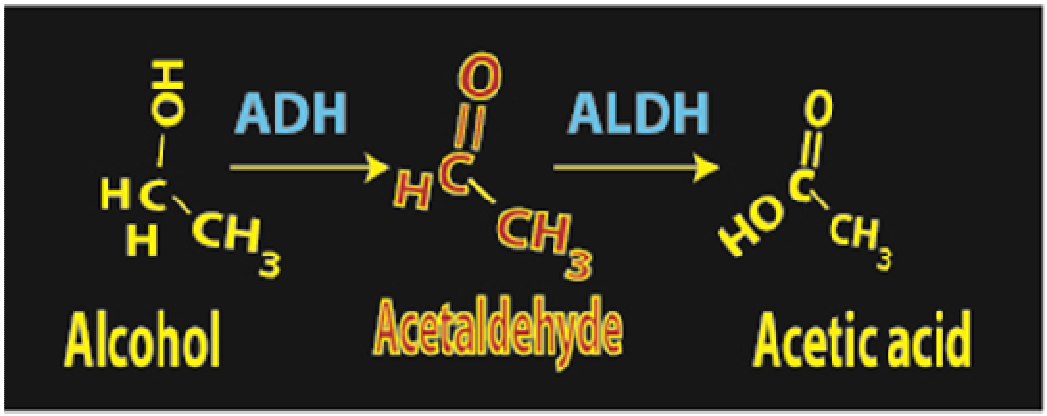

Figure 2a (Top). A major pathway for detoxification of alcohol requires two enzymes, alcohol dehydrogenase (ADH) and then aldehyde dehydrogenase (ALDH). Alcohol dehydrogenase produces acetaldehyde, which is a mutagen and carcinogen. Variations in genes encoding some detoxification enzymes are well known to influence the susceptibility to alcohol related carcinogenesis.

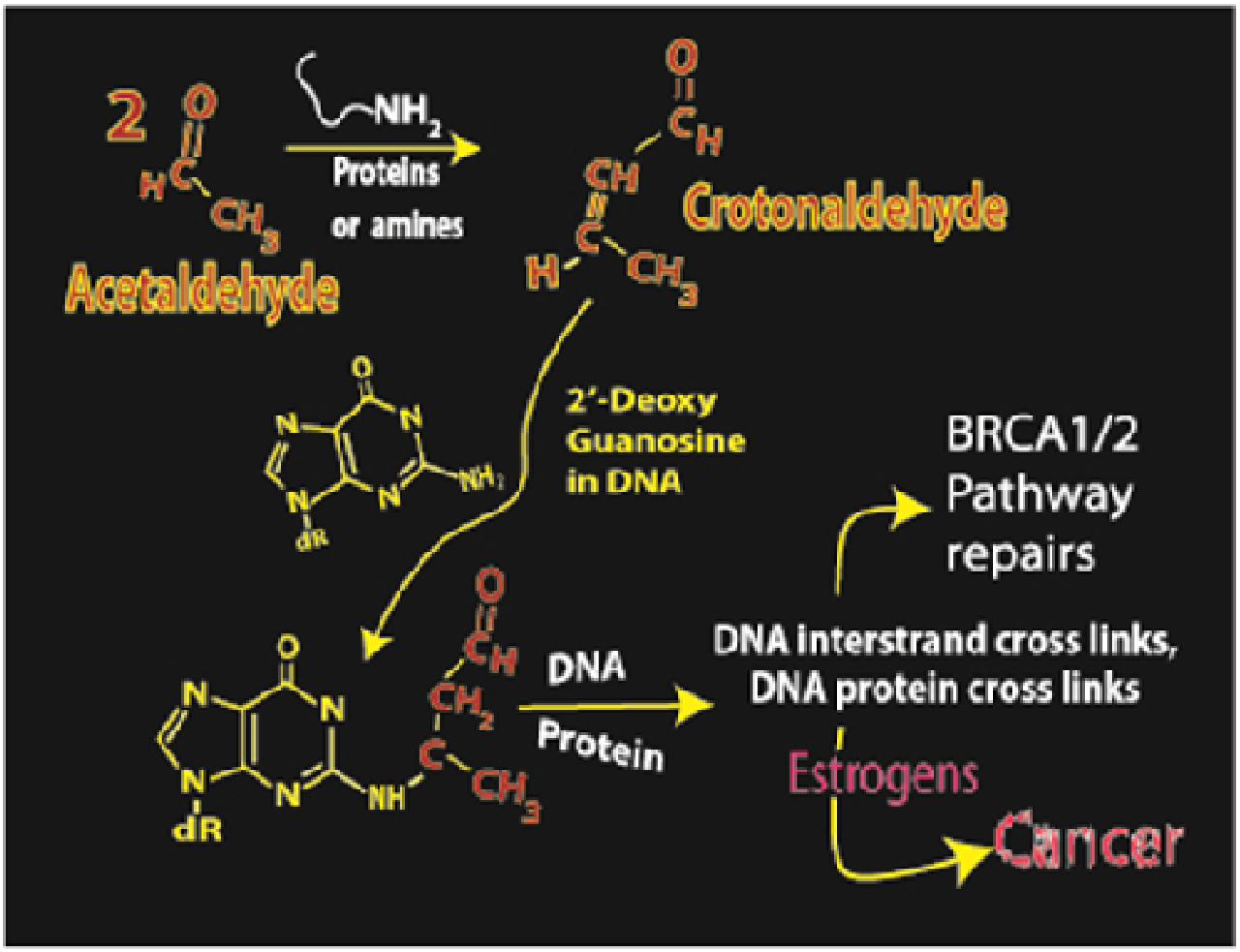

Figure 2b. One mechanism or carcinogenesis. Acetaldehyde that is ingested or escapes alcohol detoxification pathways can form crotonaldehyde, a reaction catalyzed by amine groups from proteins or naturally occurring amines. Crotonaldehyde is genotoxic, mutagenic and carcinogenic and can be derived from beer, wine and liquor. Reactions with deoxyguanosine in DNA produce DNA interstrand cross links and DNA protein cross links (Theruvathu et al., 2005). Some of these lesions require repairs by BRCA1/2 pathways. Inherited mutations for proteins within BRCA1/2 pathways disable cross link repairs and can lead to cancer in exposed organs. Excessive alcohol/acetaldehyde can also generate reactive oxygen species leading to other cross links. Effects of estrogens add to cancer risks.

Increased risks for AML extend to other hereditary gene mutations within the model pathway in Figure 3. Hereditary Fanconi and ATM gene mutations associate with very high risks for AML. ATM deficits increase the frequency of translocations involving the multi-lineage leukemia gene (MLL). In contrast to most chromosome translocations in leukemia, a strong non-homologous end joining repair signature exists at all the chromosome translocation breakpoint junctions that involve the MLL gene (Table 2). Non-homologous end joining is an error tolerant repair mechanism that remains functional when high fidelity repairs are crippled by BRCA1/2 pathway mutation (Venkitaraman, 2003).

Statistical evidence from epidemiologic studies further supports links between myeloid leukemia and pathways containing BRCA1/2. Most epidemiologic studies show deficits in BRCA1/2 pathways associate with increased risks for myeloid leukemia (see supplementary Table in Appendix).

Myeloid leukemia associates with formaldehyde and acetaldehyde as well as BRCA1/2 mutations

Formaldehyde is now a proven cause of human myeloid leukemia. Myeloid leukemia was a prototype in formulating the cancer stem cell theory. As measured by increases in characteristic stem cell pathways, formaldehyde activates and damages stem cells during carcinogenesis (Andersen et al., 2010; Friedenson, 2011). Sophisticated mass spectrometry has shown that

Table 2. Connections between BRCA1/2 containing pathways and leukemias

Leukemia	Evidence for connections to BRCA1/2 pathway
AML	NPM is a substrate for BRCA1-BARD1. BRCA1-BARD1 co-expression in cells stabilizes NPM against degradation (Sato et al., 2004). The BRCA1-BARD1 heterodimer catalyzes the ubiquitylation of NPM in vitro and in vivo. In AML in China, where pollution is high, NPM is frequently lost (Wang et al., 2010). The NPM1 locus on chromosome 5 is part of a region that modifies breast cancer risk in BRCA1 related breast cancers. Familial AML is linked to the loss of the long arm of chromosome 5 which includes the NPM gene on chromosome 5q33–34. 5q33-34 contains one or more genes that modify breast cancer risk in BRCA1 mutation carriers (Nathanson et al., 2002). Loss of genes on chromosome 4 (Tirkkonen et al., 1997) also implicates formaldehyde and alcohol/acetaldehyde because chromosome 4q encodes all the ADH genes. ATM mutations increase the incidence of translocations at 11q23 involving the multi-lineage leukemia gene (Zhang and Rowley, 2006). Hypermethylation of promoter regions of either FANCC or FANCL (which encode components of the pathway in Fig 3.) is found in sporadic acute leukemia (Hess et al., 2008). Potential heterozygous BRCA1/BRCA2 mutations increased risks for myeloid leukemias by at least 3 fold in 7 studies and by at least 50% in 12 (see supplementary Table). (Friedenson, 2011). In Fanconi anemia patients, summary relative risks for AML were 703.3 [363.7–1354.5] (Friedenson, 2007). The model in Fig 3 shows how Fanconi proteins are required for activities of BRCA1 and BRCA2. Even in homozygous Fanconi anemia, there are few stringent genotype–phenotype connections, suggesting influences of other genes and environmental factors. Children born with defects on both chromosomes affecting genes encoding BRCA2 (Fanconi protein D-1) or affecting FANCN (PALB2) genes are especially prone to develop AML. Homozygous or biallelic mutations in BRCA2 (Fanconi protein D1) carry a 79% risk of leukemia (primarily AML) by age 10 (Alter, 2003). Fanconi protein A is lost in a subset of sporadic AML. ATM deficits increase the frequency of translocations involving MLL which occur in approximately 15% of patients with AML or ALL, constituting a WHO subtype. Over 70 MLL translocations have been reported all showing a lack of BRCA1/2 pathway mediated repairs (Zhang and Rowley, 2006)

Table 2 cont.

AML, CML	BCR/ABL fusion protein inhibits the formation of nuclear FANCD2 foci (which require the pathway in Fig 3) (Valeri et al., 2010).
Myeloid leukemias	Myeloid leukemias frequently lose chromosome 17 [monosomy 17] containing BRCA1 and p53 (Zhu et al., 2008).
Acute promyelocytic leukemia (APL)	PML fusion protein with retinoic acid receptor causes loss of PML protein function. PML is essential for proper localization of RAD51 in nuclear foci and efficient homology directed repair. PML also participates in localizing BRCA1. PML is critical for forming nuclear bodies with important functions in transcription, apoptosis, DNA repair and antiviral responses. BRCA1 colocalizes with these nuclear bodies. Loss of PML function or expression is associated with APL and progression of some solid tumors. PML helps regulate BRCA1 and PML is critical for proper localization of the essential repair protein Rad51 (Fig 3) in nuclear foci, and for efficient homology-directed repair. In cells expressing SV40 large T antigen, Rad51 foci depend on PML (Boichuk et al., 2011) . Genes involved in DNA replication/recombination/repair were consistently down regulated in APL; BRCA1 and RAD51 expression was reduced 3 to 14.3 fold (Casorelli et al., 2006)

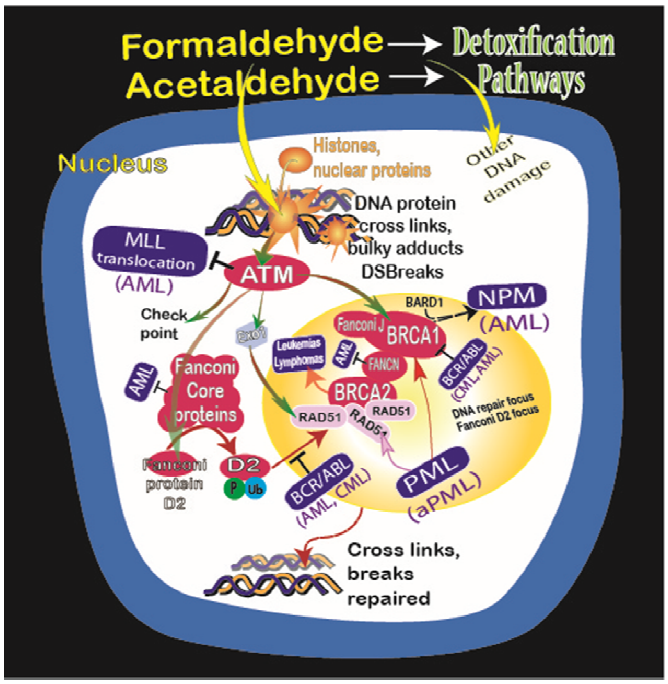

Figure 3. BRCA1/2 in DNA damage repair pathways showing probable links to myeloid leukemias. BRCA1 and BRCA2 participate in pathways to correct DNA cross links and double strand breaks caused by formaldehyde, acetaldehyde and other agents that cause bulky addition products or DNA cross-links. DNA damage is more likely if the carcinogen escapes metabolic detoxification pathways (Figs. 1, 2a). Hereditary inactivation of a Fanconi gene causes Fanconi anemia, inactivation of the ATM gene causes ataxia-telangiectasia (A-T), and inactivation of BRCA1 or BRCA2 associates with hereditary breast/ovarian cancers. Proteins encoded by genes related to these hereditary cancer conditions are colored red. The dark boxes indicate proven links to myeloid leukemia. The BRCA1-BARD1 complex is required to activate the NPM gene, which is lost in a major subset of AML. In AML and CML, the BCR/ABL protein interferes with the formation of nuclear Fanconi protein D2 repair foci. The PML protein is required for Rad51 repair focus formation. Family history of leukemia increases risk for breast cancer. Chromosome 13q (encoding BRCA2) is deleted in a subgroup of leukemias and lymphomas. Multiple other pathways participate in repairing DNA damage and members of the pathway shown have been implicated in coordinating repairs.

Table 3. Epidemiologic studies of acetaldehyde/alcohol exposure in early onset breast cancers

Patients	Effects on breast cancer risks
>1000 breast cancer patients and fast alcohol metabolizers based on ADH genotype (Terry et al., 2006)	Premenopausal breast cancer risk OR=2.9 [1.2-7.1] for 1-2 drinks per day
ADH1C genotype in 117 moderate alcohol consumers with breast cancer (Coutelle et al., 2004). The ADH1C*1 allele encodes a rapid alcohol metabolizing enzyme that produces increased acetaldehyde levels.	Women with the ADH1C*1,1 genotype had 1.8 times more risk for breast cancer than those with another genotype. This allele was significantly more frequent in moderate alcohol consumers with breast cancer vs. age-matched alcoholic controls without cancer (62% vs. 41.9%). Other combinations of alcohol metabolizing alleles may also predispose to cancers (Lachenmeier et al., 2009)
Daily drinkers who were first degree relatives of breast cancer probands(Vachon et al., 2001)	RR=2.45 [1.2-5.02]
134 premenopausal, 181 postmenopausal breast cancer patients with an efficient form of ADH vs. controls with similar alcohol intakes(Freudenheim et al., 1999)	Premenopausal breast cancer risk OR=3.6 [1.5-8.8]
Females age >=20 diagnosed with breast cancer and tested for BRCA1/2 mutations. 283 BRCA1 and 204 BRCA2 positive cases(Moorman et al., 2010)	BRCA1/2 mutation carriers who consumed alcohol got breast cancer at ages 1.7-2.9 years younger than those who did not drink
Females age 9-15 in 1996. In 2005-2007 surveys, 67 of 6888 women age 18-27 reported benign breast disease and 6741 reported no benign breast disease (Berkey et al., 2011). Benign breast disease is a well-documented breast cancer risk factor	Young women whose mothers or aunts had breast cancer were more likely to have benign breast disease (OR=2.34), as were those with maternal benign breast disease (OR=1.59). Adolescents with a family history of breast cancer who consumed 7 alcohol drinks/wk. doubled their benign breast disease risk (OR=2.28)
Women with a family history of breast cancer 40-80 and alcohol use (Yaghjyan et al., 2012).	OR=3.7 [1.7-7.8] was over twice as high as OR for women without a family history

5-formylcytosine is a constituent of mammalian embryonic stem cell DNA.

Rats fed either acetaldehyde or formaldehyde in their drinking water have very high incidence of leukemias and lymphomas (Soffritti et al., 2002a; Soffritti et al., 2002b). Model studies involving acetaldehyde show oxidative DNA damage via the metabolism of ethanol by pathways mediated by ADH and aldehyde dehydrogenase (ALDH) isoenzymes. Searches of human epidemiologic studies found associations between alcohol and myeloid leukemias. Alcohol consumption during pregnancy caused a 56% increased risk for childhood AML (Latino-Martel et al., 2010). In children born to women who drank during the second or third trimester of pregnancy,

odds ratios for leukemia were 10.48 [2.8 - 39.1] (Shu et al., 1996).

Acetaldehyde and formaldehyde as opportunistic carcinogens in hereditary breast cancers

In Sprague-Dawley rats, the number of malignant mammary tumors increased in all females who consumed acetaldehyde in their drinking water. Some malignant mammary tumors also occurred in treated males (Soffritti et al., 2002a; Soffritti et al., 2002b). An exaggerated blood acetaldehyde response after giving ethanol to pregnant rats begins a much larger alteration during lactation. At the end of pregnancy, there is a 4-fold increase in acetaldehyde above non-pregnant values after an intragastric dose of 3 g/kg ethanol. During gestational days 1-17, the levels did not differ. After delivery, the exaggerated acetaldehyde response to ethanol increased, producing acetaldehyde concentrations 15-fold greater than in non-lactating controls (Gordon et al., 1985).

Rats have the ability to activate ethanol to acetaldehyde in mammary tissue (Castro et al., 2003). Despite past controversy, there is clear and convincing evidence that acetaldehyde occurs in human breast milk (Adachi J, 1991; Wako). Breast tissue contains alcohol and aldehyde dehydrogenases and ADH is highly expressed in normal human mammary epithelium. Human breast tissue can convert ethanol to acetaldehyde and can produce free radicals as well (Triano et al., 2003).

Acetaldehyde/alcohol and breast cancer risks in potential mutation carriers

In the general population more than 100 published studies find that drinking alcohol increases breast cancer risk. Seven studies in Table 3 support an association between alcohol consumption and early onset breast cancers, including those in BRCA1/2 mutation carriers. Women who were mother, sister, or daughters of the women with breast cancer and were daily drinkers had twice the increased risk of breast cancer compared to those who never drank. Women who had married into the family but were not blood relatives had no higher risk of breast cancer if they were daily drinkers than if they never drank.(Vachon et al., 2001).

Three studies in Table 3 concluded that the genotype encoding the ADH enzyme (that initially converts alcohol to acetaldehyde) plays a role in early onset breast cancer, presumably by increasing acetaldehyde accumulation. Background levels of acetaldehyde from diet and the environment can be so high that they confound risk estimates (see Discussion). However, two additional results in Table 3 show that women who are potential or known mutation carriers have higher risks for breast cancer or get breast cancer years sooner if they consume alcohol. Table 3 also cites a study of young women with benign breast disease, a risk factor for breast cancer. Young women with a family history of breast cancer or benign breast disease who consumed alcohol doubled their risk of benign breast disease. Evidence from breast cancer clusters on Long Island, New York also suggests that alcohol may increase risk in mutation carriers. Women who lived in this area and developed breast cancer were more likely to use alcohol and to have risk factors associated with BRCA mutations. BRCA mutation related risk factors included Ashkenazi Jewish heritage and a family history of breast cancer.

In normal women, acetaldehyde becomes a carcinogen when its levels exceed 60 micromolar and this is easily achieved by some components of the diet (Salaspuro, 2009). Acetaldehyde accumulates in mammary tissue following a single dose of oral ethanol (Seitz, 2012) and has been linked to the type of chromosome aberrations associated with genetic deficits in mutation carriers and to the production of reactive oxygen species which are known to generate some of these aberrations (Wright et al., 1999).

Mammographic breast density reflects the amount of fat, connective, and epithelial tissue in the breast (Warren, 2004). Light areas on the mammogram represent the fibrous and glandular tissues in the breast, whereas the dark areas are primarily fat. The density is a consequence of the hormonal environment and underlying genetics regulating epithelial cell proliferation (Warren, 2004; Yaghjyan et al., 2012) Alcohol consumption was positively associated with breast density and the association was strongest in women with a family history of breast cancer (Table 3). Acetaldehyde can cause hyper proliferative changes in epithelial cells. Epithelia from acetaldehyde treated rats are substantially thicker than from control rats (Homann et al., 1997).

In a human mammary epithelial cell line, ethanol inhibits removal of DNA adducts formed by the carcinogen benzopyrene (Singletary et al., 2004). Hormones bring pieces of chromosomes into close proximity and may increase risks for gene rearrangements. In premenopausal women, alcohol may associate with higher blood estrogen levels. Adding risks from BRCA1/2 pathway mutations to those associated with variations in alcohol metabolism and estrogen elevation may further increase cancer risks from alcohol use.

Formaldehyde and breast cancer risks

In contrast to evidence linking alcohol and acetaldehyde to breast cancer, publications finding links to formaldehyde are scarce. However, searches retrieved a toxicological study in Sprague-Dawley rats treated with

formaldehyde or methyl alcohol, a precursor (see Figure 1). The number of malignant mammary tumors increased in females treated with 100 mg/L formaldehyde or methyl alcohol alone. Some malignant mammary tumors were also found in treated males (Soffritti et al., 2002a).

Formaldehyde in the environment has been positively associated with human breast cancer risk (Coyle et al., 2005). Greater numbers of DNA-protein cross-links were found in the white cells of breast cancer patients than in matched controls (Wu et al., 2002). Higher activity of cytosolic aldehyde dehydrogenase (ALDH1) in BRCA1 breast stem cells and hematopoietic cancer stem cells suggests less ability to remove aldehydes and greater susceptibility to mutagenic effects.

BRCA1/2 mutation carriers even from the same family have widely varying cancer risks

There is no single breast or ovarian cancer risk associated with BRCA1/2 carrier status. Defective BRCA genes increase risks for cancers in organs other than breast and ovary but individual risks again differ greatly. For example, inheritance of the same mutation in the same family caused widely different cancer phenotypes. One sister had multiple cancers while her sister with the same BRCA genotype was unaffected at 65 years of age. (Smith et al., 2008). The literature also shows many further examples of differing cancer histories within the same family (Chung et al., 2007). These results implicate an increased susceptibility in organs exposed to environmental carcinogens and/or deficits in additional genes (Friedenson, 2010b).

DISCUSSION

The results here provide links between cancers associated with BRCA1/2 genetic deficiencies and chemical carcinogens such as formaldehyde and acetaldehyde that require normal BRCA1/2 genes for repair. Acetaldehyde and formaldehyde cause DNA cross links and DNA breaks resembling DNA damage found in BRCA1/2 deficient cancers. Results here support the idea that these are opportunistic carcinogens that can exploit inherited deficits in BRCA1/2 pathways. Cells and stem cells are targeted for cancer in exposed cells. Inherited differences in the intrinsic ability to metabolize alcohol or to detoxify other carcinogens would presumably further contribute to determining where cancer occurs.

Exposure is likely and widespread

Billions of pounds of formaldehyde and acetaldehyde are produced each year and human exposure is widespread (Friedenson, 2011; Lachenmeier et al., 2009; Salaspuro, 2009). Formaldehyde has been illegally used as a food preservative; acetaldehyde forms during alcohol metabolism and high levels pre-exist in some alcoholic beverages and in some foods. High level exposure occurs in people who are heavy drinkers or binge drinkers.

Most beverages and foodstuffs produced or preserved by fermentation and considered to be non-alcoholic may, in fact, contain small amounts of ethanol and high, mutagenic (>100 mM) concentrations of acetaldehyde. These include dairy products (e.g. yogurts), fermented soy products (e.g. soy sauces), tofu products, fermented vegetables (e.g. Chinese pickles and kimchi), vinegar and homemade beers and meads. Many fruits, e.g. some apples, may have their own metabolic pathways for acetaldehyde production. In the above products, acetaldehyde concentrations can reach 3500 mM and ethanol can reach 300 mM, both well above mutagenic levels. Poor oral hygiene and alcohol intolerant genotypes amplify cancer risks. Worldwide screening of thousands of foods for dangerously high acetaldehyde levels has already been advocated (Salaspuro, 2009). Dietary and environmental acetaldehyde could easily confound epidemiologic studies of breast cancer risks vs. alcohol consumption and alcohol metabolism genotypes.

Acetaldehyde exposure in man is indisputably cumulative. This concept is also supported by a recent large-scale epidemiological survey demonstrating a supra-multiplicative combined risk for esophageal cancer among alcohol and tobacco consumers, who are low ADH1B and ALDH2-deficient carriers, the highest adjusted odds ratio being as high as 382.3 (Lee et al., 2008; Salaspuro, 2009). Elevated risks for digestive tract cancers were found in some studies of BRCA1/2 mutation carriers (Friedenson, 2005) and a connection to pancreatic cancer is well known. Moreover, other alleles or combinations of alleles contribute to cancer risks in various populations (Lachenmeier et al., 2009). Fortunately, acetaldehyde exposure may be decreased or even totally abolished by using special medical devices that slowly release L-cysteine (Salaspuro, 2009).

Activities of detoxification pathways may further stratify risks

Genetic polymorphisms that affect activities of detoxification enzymes and behavior are potential lurking variables in assigning cancer risks from formaldehyde and acetaldehyde. For example, some ALDH2 genotypes in Asian BRCA carriers may impair acetaldehyde metabolism. This increases cancer risks but makes acetaldehyde more unpleasant to consume.

Activity of dehydrogenases, oxidoreductases, and cytochrome P450 enzymes (Seitz and Becker, 2007;

Seitz and Stickel, 2007) might further stratify risks among mutation carriers. If so, then, broader genetic testing may be helpful.

Seven studies in Table 3 report alcohol consumption increases breast cancer risk in rather restricted populations of potential BRCA1/2 mutation carriers. The results are consistent with current understanding of alcohol and breast cancer risks, with animal studies, with mechanisms of BRCA related carcinogenesis, and with model studies; so confounding effects may be more limited in restricted case control comparisons.

Acetaldehyde or formaldehyde need not be responsible for all types of cancers in BRCA1/2 mutation carriers. But again the cancer target is probably determined by exposure to opportunistic carcinogens. Genomic analyses of 316 high grade ovarian cancers showed that homologous recombination is defective in about half of ovarian cancers and that p53 is mutated in 96% (Cancer Genome Atlas Research, 2011). Much ovarian cancer begins in the fimbriae - fingerlike projections terminating the Fallopian tubes where the ovum released from the ovary is collected. Tubal ligation protects both mutation carriers and non-carriers against ovarian cancer. Tubal ligation commonly removes sections of tubes between the uterus and fimbria, presumably preventing chronic infection/inflammation from reaching the fimbriae/ovaries. There are also other associations between chronic unresolved infection/ inflammation and fimbrial-ovarian cancer (Friedenson, 2010a).

After these results were obtained, other inherited cancer conditions were quickly tested against the conclusions. This test showed that the phenomenon of increased sensitivity to environmental carcinogens such as formaldehyde and acetaldehyde are probably representative of a broad general phenomenon. Common findings are abnormal sensitivity to radiation, to chronic infections associated with cancer, and/or to chemical carcinogens. The genetic disease xeroderma pigmentosum is a well-known example of this increased environmental sensitivity. The inherited genetic deficit greatly amplifies cancer risks from sunlight, causing this radiation to become an opportunistic carcinogen. Risks are higher for homozygotes but are also increased for heterozygotes. Further studies of relationships among genetic deficiencies and environmental carcinogens are needed.

Treatment related risk factors for myeloid leukemia

Inherited BRCA1/2 pathway deficits probably cause increased sensitivity to additional carcinogens that become opportunistic. Increased risks for other cancers related to known chronic infectious agents support this possibility (Friedenson, 2010a). Some chemotherapy for BRCA1/2 related breast cancers can also behave as opportunistic carcinogens. Infection prophylaxis with myeloid growth factors during chemotherapy might increase risks still further. The lack of data separating risk factors from treatment risks makes it difficult to determine risks associated with treatment.

Radiation therapy

γ–Radiation generates DNA damage that requires BRCA1/2 pathway mediated repairs so BRCA1/2 patients may be unduly susceptible to radiation related cancers (Friedenson, 2000) including leukemia and breast cancer. Evidence is emerging that chest x-rays, mammography, CT scans and radiation therapy may become opportunistic carcinogens in BRCA1/2 mutation carriers.

CONCLUSIONS

Several types of DNA damage associated with hereditary BRCA1/2 cancers are the same types of DNA damage caused by the carcinogens formaldehyde and acetaldehyde. BRCA1/2 mutation carriers have elevated risks for cancers known to be associated with formaldehyde and acetaldehyde. Both carcinogens activate BRCA1/2 pathways; both carcinogens cause the same types of DNA damage found in BRCA1/2 related cancers; both carcinogens increase risks for cancers associated with BRCA1/2 deficiencies. Breastfeeding transmits acetaldehyde to infants and at least seven studies link acetaldehyde to early onset breast cancer, despite potential confounders. There are known ways to modify risks from these and other opportunistic carcinogens such as radiation in BRCA mutation carriers. Some hereditary cancers might be preventable by compensating for genetic deficits.

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REFERENCES

Abraham J, Balbo S, Crabb D, Brooks PJ (2011). Alcohol metabolism in human cells causes DNA damage and activates the Fanconi anemia-breast cancer susceptibility (FA-BRCA) DNA damage response network. Alcoholism, Clinical and Exp. Res. 35:2113-2120.

Adachi JMY, Naito T, Ogawa Y, Uetani Y, Ninomiya I (1991). Identification of tetrahydro-beta-carboline-3-carboxylic acid in foodstuffs, human urine and human milk. J. Nutr. 121:646-652.

Alter BP (2003). Cancer in Fanconi anemia, 1927-2001. Cancer 97:425-440.

Andersen ME, Clewell HJ, 3rd, Bermudez E, Dodd, DE, Willson GA, Campbell JL,Thomas RS (2010). Formaldehyde: integrating dosimetry, cytotoxicity, and genomics to understand dose-dependent transitions for an endogenous compound. Toxicological sciences :an official journal of the Society of Toxicology 118: 716-731.

Ashworth A (2008). A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. J. Clin. Oncol. *26*:3785-3790.

Berkey CS, Tamimi RM, Rosner B, Frazier AL, Colditz GA (2011). Young women with family history of breast cancer and their risk factors for benign breast disease. Cancer. Nov. 14, 2011 doi: 10.1002/cncr.26519

Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK (2000). The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. The J. Biol. Chem. *275*: 23899-23903.

Bijron JG, van der Groep P, van Dorst EB, Seeber LM, Sie-Go DM, Verheijen RH, van Diest PJ (2011). Promoter hypermethylation patterns in Fallopian tube epithelium of BRCA1 and BRCA2 germline mutation carriers. Endocrine-related cancer.

Boichuk S, Hu L, Makielski K, Pandolfi PP, Gjoerup OV (2011). Functional connection between Rad51 and PML in homology-directed repair. PloS one *6*, e25814.

Cancer Genome Atlas Research, N. (2011). Integrated genomic analyses of ovarian carcinoma. Nature *474*:609-615.

Casorelli I, Tenedini E, Tagliafico E, Blasi MF, Giuliani A, Crescenzi M, Pelosi E, Testa U, Peschle C, Mele L, Diverio D, Breccia,M, Lo-Coco F, Ferrari S, Bignami M (2006). Identification of a molecular signature for leukemic promyelocytes and their normal counterparts: Focus on DNA repair genes. Leukemia: official journal of the Leukemia Society of America, Leukemia Research Fund, UK *20*, 1978-1988.

Castro GD, Delgado de Layno AM, Costantini MH, Castro JA (2003). Rat breast microsomal biotransformation of ethanol to acetaldehyde but not to free radicals: its potential role in the association between alcohol drinking and breast tumor promotion. Teratogenesis, carcinogenesis, and mutagenesis *Suppl. 1*:61-70.

Cho YJ, Wang H, Kozekov ID, Kurtz AJ, Jacob J, Voehler M, Smith J, Harris TM, Lloyd RS, Rizzo CJ, Stone M (2006). Stereospecific formation of interstrand carbinolamine DNA cross-links by crotonaldehyde- and acetaldehyde-derived alpha-CH3-gamma-OH-1,N2-propano-2'-deoxyguanosine adducts in the 5'-CpG-3' sequence. Chem. Res. Toxicol. *19*:195-208.

Chung DC, Yoon SS, Lauwers GY, Patel D (2007). Case records of the Massachusetts General Hospital. Case 22-2007. A woman with a family history of gastric and breast cancer. The N. Engl. J. Med. *357*: 283-291.

Coutelle C, Hohn B, Benesova M, Oneta CM, Quattrochi P, Roth HJ, Schmidt-Gayk H, Schneeweiss A, Bastert G, Seitz HK (2004). Risk factors in alcohol associated breast cancer: alcohol dehydrogenase polymorphism and estrogens. International journal of oncology *25*:1127-1132.

Coyle YM, Hynan LS, Euhus DM, and Minhajuddin, A.T. (2005). An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. Breast Cancer Res. Treatment *92*:107-114.

Daniel FI, Rivero ER, Modolo F, Lopes TG, Salum FG (2010). Immunohistochemical expression of DNA methyltransferases 1, 3a and 3b in oral leukoplakias and squamous cell carcinomas. Archives of oral biology *55*, 1024-1030.

De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg, H, Drake CG, Nakai Y, Isaacs WB, Nelson WG. (2007). Inflammation in prostate carcinogenesis. Nature Rev. Cancer. *7*:256-269.

Fang JL, Vaca CE (1997). Detection of DNA adducts of acetaldehyde in peripheral white blood cells of alcohol abusers. Carcinogenesis *18*:627-632.

Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Graham S, Marshall JR, Muti P, Laughlin R, Nemoto T, Harty LC, Crits G, Chan A. Shields P (1999). Alcohol dehydrogenase 3 genotype modification of the association of alcohol consumption with breast cancer risk. Cancer causes & control: CCC *10*, 369-377.

Friedenson B (2000). Is mammography indicated for women with defective BRCA genes? Implications of recent scientific advances for the diagnosis, treatment, and prevention of hereditary breast cancer. MedGenMed : Medscape general medicine *2*, E9.

Friedenson B (2005). BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. MedGenMed : Medscape general medicine *7*:60.

Friedenson B (2007). The BRCA1/2 pathway prevents hematologic cancers in addition to breast and ovarian cancers. BMC cancer *7*:152.

Friedenson B (2010a). Inflammatory processes inordinately increase tissue specific cancer risks in carriers of mutations in BRCA1,

BRCA2, ATM or Fanconi anemia genes. J. Med. Med. Sci. *1*:356-370.

Friedenson B (2010b). A theory that explains the tissue specificity of BRCA1/2 related and other hereditary cancers. J. Med. Med. Sci. *1*:372-383.

Friedenson B (2011). A common environmental carcinogen unduly affects carriers of cancer mutations: carriers of genetic mutations in a specific protective response are more susceptible to an environmental carcinogen. Medical hypotheses *77*: 791-797.

Garro AJ, McBeth DL, Lima V, Lieber CS (1991). Ethanol consumption inhibits fetal DNA methylation in mice: implications for the fetal alcohol syndrome. Alcoholism, Clinical and Exp. Res. *15*:395-398.

Gordon BH, Baraona E, Miyakawa H, Finkelman F, Lieber CS (1985). Exaggerated acetaldehyde response after ethanol administration during pregnancy and lactation in rats. Alcoholism, clinical and Exp. Res. *9*:17-22.

Gosepath J, Brieger J, Muttray A, Best S, Pourianfar M, Jung D, Letzel S, Mann WJ (2006). mRNA induction and cytokine release of inflammatory mediators during in vitro exposure of human nasal respiratory epithelia to acetaldehyde. Inhalation toxicol. *18*:1083-1090.

Hess CJ, Ameziane N, Schuurhuis GJ, Errami A, Denkers F, Kaspers GJ, Cloos J, Joenje H, Reinhardt, D, Ossenkoppele GJ, Zwaan C, Waisfisz Q (2008). Hypermethylation of the FANCC and FANCL promoter regions in sporadic acute leukaemia. Cellular oncology: the official journal of the International Society for Cellular Oncology *30*, 299-306.

Homann N, Karkkainen P, Koivisto T, Nosova T, Jokelainen K, Salaspuro M (1997). Effects of acetaldehyde on cell regeneration and differentiation of the upper gastrointestinal tract mucosa. Journal of the National Cancer Institute *89*:1692-1697.

Huen MS, Sy SM, Chen J (2010). BRCA1 and its toolbox for the maintenance of genome integrity. Nature reviews Molecular cell biology *11*:138-148.

Ishida E, Nakamura M, Ikuta M, Shimada K, Matsuyoshi S, Kirita T, Konishi N (2005). Promotor hypermethylation of p14ARF is a key alteration for progression of oral squamous cell carcinoma. Oral oncology *41*:614-622.

Ishikawa H, Ishikawa T, Yamamoto H, Fukao A, Yokoyama K (2007). Genotoxic effects of alcohol in human peripheral lymphocytes modulated by ADH1B and ALDH2 gene polymorphisms. Mutat. Res. *615*: 134-142.

Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A (2001). Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nature genetics *29*: 418-425.

Lachenmeier DW, Kanteres F, Rehm J (2009). Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. Addiction *104*:533-550.

Latino-Martel P, Chan DS, Druesne-Pecollo N, Barrandon E, Hercberg S, Norat T (2010). Maternal alcohol consumption during pregnancy and risk of childhood leukemia: systematic review and meta-analysis. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology *19*:1238-1260.

Lee CH, Lee JM, Wu DC, Goan YG, Chou SH, Wu IC, Kao EL, Chan TF, Huang MC, Chen PS, Lee C, Huang C, Huang H, Hu C, Hung Y, Wu M (2008). Carcinogenetic impact of ADH1B and ALDH2 genes on squamous cell carcinoma risk of the esophagus with regard to the consumption of alcohol, tobacco and betel quid. International journal of cancer Journal international du cancer *122*:1347-1356.

Liu Q, Yang L, Gong C, Tao G, Huang H, Liu J, Zhang H, Wu D, Xia B, Hu G, Wang K, Zhuang Z. (2011). Effects of long-term low-dose

formaldehyde exposure on global genomic hypomethylation in 16HBE cells. Toxicol. Lett. *205*: 235-240.

Lu K, Ye W, Zhou L, Collins LB, Chen X, Gold A, Ball, LM, Swenberg JA (2010). Structural characterization of formaldehyde-induced cross-links between amino acids and deoxynucleosides and their oligomers. J. Am. Chem. Soc. *132*:3388-3399.

Machida K, Cheng KT, Lai CK, Jeng KS, Sung VM, Lai MM (2006). Hepatitis C virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. J. Virol. *80*:7199-7207.

Mao X, Boyd LK, Yanez-Munoz RJ, Chaplin T, Xue L, Lin D, Shan L, Berney DM, Young BD, Lu YJ (2011). Chromosome rearrangement associated inactivation of tumour suppressor genes in prostate cancer. American journal of cancer research *1*:604-617.

Marietta C, Thompson LH, Lamerdin JE, Brooks PJ, (2009). Acetaldehyde stimulates FANCD2 monoubiquitination, H2AX phosphorylation, and BRCA1 phosphorylation in human cells in vitro: implications for alcohol-related carcinogenesis. Mutat Res *664*:77-83.

Maul GG, Jensen DE, Ishov AM, Herlyn M, Rauscher, FJ, 3rd (1998). Nuclear redistribution of BRCA1 during viral infection. Cell growth & differentiation: the molecular biology journal of the American Association for Cancer Research *9*:743-755.

Moorman PG, Iversen ES, Marcom PK, Marks JR, Wang F, Kathleen Cuningham Consortium for Research into Familial Breast, C, Lee E, Ursin G, Rebbeck TR, Domchek SM, Arun B, Susswein L, Garber J, Visvanathan K, Griffin C, Sutphen R, Brzosowicz J, Gruber S, Finkelstein D, Schildkraut J (2010). Evaluation of established breast cancer risk factors as modifiers of BRCA1 or BRCA2: a multi-center case-only analysis. Breast cancer research and treatment *124*:441-451.

Moro L, Arbini AA, Yao JL, di Sant'Agnese PA, Marra, E, Greco M (2008). Loss of BRCA2 promotes prostate cancer cell invasion through up-regulation of matrix metalloproteinase-9. Cancer Sci. *99*:553-563.

Nakano T, Katafuchi A, Matsubara M, Terato H, Tsuboi T, Masuda T, Tatsumoto T, Pack SP, Makino K, Croteau DL, , Croteau D, Van Houten B, Iijima K, Tauchi H, Ide H (2009). Homologous recombination but not nucleotide excision repair plays a pivotal role in tolerance of DNA-protein cross-links in mammalian cells. The Journal of biological chemistry *284*:27065-27076.

Nathanson KL, Shugart YY, Omaruddin R, Szabo C, Goldgar D, Rebbeck TR, Weber BL (2002). CGH-targeted linkage analysis reveals a possible BRCA1 modifier locus on chromosome 5q. Human molecular genetics *11*:1327-1332.

Navaraj A, Finnberg N, Dicker DT, Yang W, Matthew EM, El-Deiry WS (2009). Reduced cell death, invasive and angiogenic features conferred by BRCA1-deficiency in mammary epithelial cells transformed with H-Ras. Cancer biology & therapy *8*, 2417-2444.

Noori P, Hou SM (2001). Mutational spectrum induced by acetaldehyde in the HPRT gene of human T lymphocytes resembles that in the p53 gene of esophageal cancers. Carcinogenesis *22*:1825-1830.

Obe G, Anderson D (1987). International Commission for Protection against Environmental Mutagens and Carcinogens. ICPEMC Working Paper No. 15/1. Genetic effects of ethanol. Mutat Res *186*, 177-200.

Paget V, Lechevrel M, Sichel F (2008). Acetaldehyde-induced mutational pattern in the tumour suppressor gene TP53 analysed by use of a functional assay, the FASAY (functional analysis of separated alleles in yeast). Mutat. Res. *652*:12-19.

Salaspuro M (2009). Acetaldehyde: a cumulative carcinogen in humans. Addiction *104*:551-553.

Sato K, Hayami R, Wu W, Nishikawa T, Nishikawa H, Okuda Y, Ogata H, Fukuda M, Ohta T (2004). Nucleophosmin/B23 is a candidate substrate for the BRCA1-BARD1 ubiquitin ligase. The J. Biol. Chem. *279*:30919-30922.

Seitz HK (2012). Alcohol and breast cancer. Breast.

Seitz HK, Becker P (2007). Alcohol metabolism and cancer risk. Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism *30*:38-41, 44-37.

Seitz HK, Stickel F (2007). Molecular mechanisms of alcohol-mediated carcinogenesis. Nature Rev. Cancer *7*:599-612.

Shaham J, Bomstein Y, Gurvich R, Rashkovsky M, Kaufman Z (2003). DNA-protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. Occupational and Environ. Med. *60*:403-409.

Shaham J, Bomstein Y, Meltzer A, Kaufman Z, Palma, E, Ribak J (1996). DNA--protein crosslinks, a biomarker of exposure to formaldehyde--in vitro and in vivo studies. Carcinogenesis *17*:121-125.

Shu XO, Ross JA, Pendergrass TW, Reaman GH, Lampkin B, Robison LL (1996). Parental alcohol consumption, cigarette smoking, and risk of infant leukemia: a Childrens Cancer Group study. Journal of the National Cancer Institute *88*:24-31.

Singletary KW, Barnes SL, van Breemen RB (2004). Ethanol inhibits benzo[a]pyrene-DNA adduct removal and increases 8-oxo-deoxyguanosine formation in human mammary epithelial cells. Cancer Lett. *203*: 139-144.

Smith M, Fawcett S, Sigalas E, Bell R, Devery S, Andrieska N, Winship I (2008). Familial breast cancer: double heterozygosity for BRCA1 and BRCA2 mutations with differing phenotypes. Familial cancer. *7*: 119-124.

Soffritti M, Belpoggi F, Cevolani D, Guarino M, Padovani M, Maltoni C (2002a). Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. Annals of the New York Academy of Sciences. *982*:46-69.

Soffritti M, Belpoggi F, Lambertin L, Lauriola M, Padovani M, Maltoni C (2002b). Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Annals of the New York Academy of Sciences *982*:87-105.

Stefansson OA, Jonasson JG, Olafsdottir K, Hilmarsdottir H, Olafsdottir G, Esteller M, Johannsson, OT, Eyfjord JE (2011). CpG island hypermethylation of BRCA1 and loss of pRb as co-occurring events in basal/triple-negative breast cancer. Epigenetics : official journal of the DNA Methylation Society *6*, 638-649.

Sutcliffe S, Giovannucci E, Gaydos CA, Viscidi RP, Jenkins FJ, Zenilman JM, Jacobson LP, De Marzo AM, Willett WC, Platz EA (2007). Plasma antibodies against Chlamydia trachomatis, human papillomavirus, and human herpesvirus type 8 in relation to prostate cancer: a prospective study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology *16*:1573-1580.

Szaumkessel M, Richter J, Giefing M, Jarmuz M, Kiwerska K, Tonnies H, Grenman R, Heidemann S, Szyfter K, Siebert R (2011). Pyrosequencing-based DNA methylation profiling of Fanconi anemia/BRCA pathway genes in laryngeal squamous cell carcinoma. International journal of oncology *39*:505-514.

Terry MB, Gammon MD, Zhang FF, Knight JA, Wang Q, Britton JA, Teitelbaum SL, Neugut AI, Santella RM (2006). ADH3 genotype, alcohol intake and breast cancer risk. Carcinogenesis *27*:840-847.

Theruvathu JA, Jaruga P, Nath RG, Dizdaroglu M, Brooks PJ (2005). Polyamines stimulate the formation of mutagenic 1,N2-propanodeoxyguanosine adducts from acetaldehyde. Nucleic Acids Res. *33*:3513-3520.

Tirkkonen M, Johannsson O, Agnarsson BA, Olsson H, Ingvarsson S, Karhu R, Tanner M, Isola J, Barkardottir RB, Borg A, Kallioniemi O (1997). Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. Cancer Res. *57*:1222-1227.

Triano EA, Slusher LB, Atkins TA, Beneski JT, Gestl SA, Zolfaghari R, Polavarapu R, Frauenhoffer E, Weisz J (2003). Class I alcohol dehydrogenase is highly expressed in normal human mammary epithelium but not in invasive breast cancer: implications for breast carcinogenesis. Cancer Res. *63*:3092-3100.

Vachon CM, Cerhan JR, Vierkant RA, Sellers TA (2001). Investigation of an interaction of alcohol intake and family history on breast cancer risk in the Minnesota Breast Cancer Family Study. Cancer. *92*: 240-248.

Valeri A, Alonso-Ferrero ME, Rio P, Pujol MR, Casado JA, Perez L, Jacome A, Agirre X, Calasanz MJ, Hanenberg H, Surralles J, Prosper F, Albella B, Bueren J (2010). Bcr/Abl interferes with the Fanconi anemia/BRCA pathway: implications in the chromosomal instability of chronic myeloid leukemia cells. PloS one *5*, e15525.

Venkitaraman AR (2003). A growing network of cancer-susceptibility genes. The New England journal of medicine *348*:1917-1919.

Wako H (1953). The isolation and identification of methyl glyoxal from human milk. III. The Tohoku journal of experimental medicine *57*:191-197.

Wang L, Xu WL, Meng HT, Qian WB, Mai WY, Tong HY, Mao LP, Tong Y, Qian JJ, Lou YJ, Chen Z, Wang Y, Jin J (2010). FLT3 and NPM1 mutations in Chinese patients with acute myeloid leukemia and normal cytogenetics. Journal of Zhejiang University Science B *11*:762-770.

Wani, N.A., Hamid, A., and Kaur, J. (2011). Alcohol-associated folate disturbances result in altered methylation of folate-regulating genes. Molecular and cellular biochemistry.

Warren R (2004). Hormones and mammographic breast density. Maturitas *49*:67-78.

Wilkins RJ, Macleod HD (1976). Formaldehyde induced DNA-protein crosslinks in Escherichia Coli. Mutat. Res. *36*:11-16.

Wright RM, McManaman JL, Repine JE (1999). Alcohol-induced breast cancer: a proposed mechanism. Free Radical Biology & Medicine *26*:348-354.

Wu FY, Lee YJ, Chen DR, Kuo HW (2002). Association of DNA-protein crosslinks and breast cancer. Mutat. Res. *501*:69-78.

Yaghjyan L, Mahoney MC, Succop P, Wones R, Buckholz J, Pinney SM (2012). Relationship between breast cancer risk factors and mammographic breast density in the Fernald Community Cohort. Br. J. cancer. *106*:996-1003.

Yu VP, Koehler M, Steinlein C, Schmid M, Hanakahi LA, van Gool AJ, West SC, Venkitaraman AR (2000). Gross chromosomal rearrangements and genetic exchange between nonhomologous chromosomes following BRCA2 inactivation. Genes & development *14*, 1400-1406.

Zhang H, Somasundaram K, Peng Y, Tian H, Zhang H, Bi D, Weber BL, El-Deiry WS (1998). BRCA1 physically associates with p53 and stimulates its transcriptional activity. Oncogene *16*:1713-1721.

Zhang L, Tang X, Rothman N, Vermeulen R, Ji Z, Shen M, Qiu C, Guo W, Liu S, Reiss B, *et* Freeman L B, Ge Y, Hubbard A E, Hua M, Blair A ,Galvan N ,Ruan X, Alter B P , Xin K X,Li S ,Moore L E, Kim S, Xie Y ,Hayes R B, Azuma M, Hauptmann M, Xiong J, Stewart P ,Li L ,Rappaport S M, Huang H, Fraumeni J F Jr, Smith M T, Lan Q (2010). Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology *19*, 80-88.

Zhang Y, Rowley JD (2006). Chromatin structural elements and chromosomal translocations in leukemia. DNA Repair *5*:1282-1297.

Zhong L, Zhu ZZ, Shen Y, Sun G, Zhao X, Zhang S, Yin X, Zhu J, Xu Z, Zhu, G (2011). Frequent Germline Mutation in the BRCA2 Gene in Esophageal Squamous Cell Carcinoma Patients from a Low-risk Chinese Population. Asian Pacific journal of cancer prevention : APJCP *12*:1771-1776.

Zhu Y, Xu W, Liu Q, Pan J, Qiu H, Wang R, Qiao C, Jiang Y, Zhang S, Fan L, Shen Y, Xue Y, Li J (2008). [Abnormalities of chromosome 17 in myeloid malignancies with complex chromosomal abnormalities]. Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese Journal of Medical Genetics *25*:579-582.

Appendix

Supplementary Table. Risks of leukemia as cancers following breast, ovarian or fallopian tube cancer in proven or potential BRCA1/2 mutation carriers

Study population and reference	Mutation test status	Risk measurement for leukemias [Confidence interval]
6 children with biallelic BRCA2 mutations (Wagner et al., 2004).	Biallelic BRCA2 mutations (compound heterozygotes)	All developed leukemia at median age 2.2 years. 4 of 6 AML patients
Review of 27 biallelic BRCA2 mutation patients (Alter, 2003).	Biallelic BRCA2 mutations (compound heterozygotes)	79% cumulative probability of leukemia (primarily AML) by age 10 years
First breast cancer age <45 in 6958 Connecticut women(Harvey and Brinton, 1985).	Potential mutation carriers eligible for mutation testing	Acute non-lymphocytic leukemia as 2nd cancer O/E=2.9 at 1-4 years and 6.4 at 5-9 years
Breast cancer patients, age 35-49 from 26,617 primary female breast cancers (Teppo et al., 1985)	Potential mutation carriers eligible for mutation testing	Subsequent leukemia (excluding CLL) RR= 3.21 p<.01
Female breast cancer surviving >= 10 years (selects 11,273 younger patients)(Ewertz and Mouridsen, 1985).	Potential mutation carriers eligible for mutation testing	Acute non-lymphocytic leukemia as a 2nd cancer RR=2.3
2813 women with 2 breast or ovarian cancers in Thames Cancer Registry(Evans et al., 2001a).	Potential BRCA1/2 mutation carriers eligible for mutation testing	Myeloid leukemia RR=5.04 [1.85-11.0]
82,520 Women with breast cancer age <=45 in 13 cancer registries (Mellemkjaer et al., 2006).	Potential mutation carriers eligible for mutation testing	Myeloid Leukemia SIR = 3.02 (2.32–3.85) Leukemia SIR = 2.16 (1.78–2.59).
2,084 Women with primary fallopian tube cancer from 13 cancer registries (Riska et al., 2007).	Probable BRCA1/2 mutation carriers.	Non-lymphoid leukemia RR=3.7 (1.0-9.4)

Appendix cont.

2nd cancer after Breast Cancer<50 from 32, 799 patients in Thames Cancer Registry (Evans et al., 2001b).	Potential BRCA1/2 mutation carriers eligible for mutation testing	Myeloid leukemias RR=2.31 [1.52-3.51]
2nd cancer after male breast cancer (Hemminki et al., 2005).	Men at high risk for being (BRCA2) mutation carriers eligible for mutation testing	Myeloid leukemia RR=3.98 [1.46 – 8.67] 1-9 yrs of follow up. RR=3.42 [1.47-6.73] for all periods
534 First degree relatives of BRCA1 probands with ovarian cancer (Risch et al., 2006).	Tested BRCA1 heterozygotes or potential carriers eligible for testing	Leukemias, lymphomas, etc. RR=3.7 (1.5 to 9.5)
7/98 multiple primary cancer families with a BRCA1 mutation and 8/98 multiple primary cancer families with a BRCA2 mutation (Shih et al., 2000).	Known BRCA1 and BRCA2 mutation carriers	20% of leukemias in the families occurred in BRCA1 and BRCA2 mutation carriers.
3678 women, 50 men. First degree relatives of BRCA2 mutation carriers or of breast or ovarian cancer patients (Consortium, 1999).	471 BRCA2 carriers, 390 non-carriers, and 2186 unknown BRCA2 carrier status	Leukemia RR= 1.12 [0.30–4.25]
11847 individuals from 699 families segregating a BRCA1 mutation (Thompson et al., 2002).	18.9% (2245) tested BRCA1 carriers, 9.3% (1106) tested negative, 71.7% (8496) untested.	Leukemia RR = 0.88 [0.37 to 2.14] p=.83
1811 male and female family members (van Asperen CJ et al., 2005).	50% probability BRCA2 mutation from 139 BRCA2 families.	Leukemia RR= 1.5 [0.5 to 3.5]
728 males & females (BRCA2). 1145 males & females (BRCA1) (Johannsson et al., 1999).	From families with an identified BRCA2 or BRCA1 mutation	Acute leukemia SMR=1.54 [0.04±8.59] (BRCA2). 1.01 [0.03±5.62] (BRCA1)

Appendix cont.

References for supplementary table

Alter, B.P. (2003). Cancer in Fanconi anemia, 1927-2001. Cancer *97*, 425-440.

Consortium, T.B.C.L. (1999). Cancer risks in BRCA2 mutation carriers. . Journal of the National Cancer Institute *91*, 1310-1316.

Evans, H.S., Lewis, C.M., Robinson, D., Bell, C.M., Moller, H., and Hodgson, S.V. (2001a). Cancer risks in women with 2 breast or ovarian cancers: clues to genetic cancer susceptibility. International journal of cancer Journal international du cancer *94*, 758-759.

Evans, H.S., Lewis, C.M., Robinson, D., Bell, C.M., Moller, H., and Hodgson, S.V. (2001b). Incidence of multiple primary cancers in a cohort of women diagnosed with breast cancer in southeast England. British journal of cancer *84*, 435-440.

Ewertz, M., and Mouridsen, H.T. (1985). Second cancer following cancer of the female breast in Denmark, 1943-80. National Cancer Institute monograph *68*, 325-329.

Harvey, E.B., and Brinton, L.A. (1985). Second cancer following cancer of the breast in Connecticut, 1935-82. National Cancer Institute monograph *68*, 99-112.

Hemminki, K., Scelo, G., Boffetta, P., Mellemkjaer, L., Tracey, E., Andersen, A., Brewster, D.H., Pukkala, E., McBride, M., Kliewer, E.V.*, et al.* (2005). Second primary malignancies in patients with male breast cancer. British journal of cancer *92*, 1288-1292.

Johannsson, O., Loman, N., Moller, T., Kristoffersson, U., Borg, A., and Olsson, H. (1999). Incidence of malignant tumours in relatives of BRCA1 and BRCA2 germline mutation carriers. European journal of cancer *35*, 1248-1257.

Mellemkjaer, L., Friis, S., Olsen, J.H., Scelo, G., Hemminki, K., Tracey, E., Andersen, A., Brewster, D.H., Pukkala, E., McBride, M.L., et al. (2006). Risk of second cancer among women with breast cancer. International journal of cancer Journal international du cancer 118, 2285-2292.

Risch, H.A., McLaughlin, J.R., Cole, D.E., Rosen, B., Bradley, L., Fan, I., Tang, J., Li, S., Zhang, S., Shaw, P.A., et al. (2006). Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. Journal of the National Cancer Institute 98, 1694-1706.

Riska, A., Pukkala, E., Scelo, G., Mellemkjaer, L., Hemminki, K., Weiderpass, E., McBride, M.L., Pompe-Kirn, V., Tracey, E., Brewster, D.H., et al. (2007). Second primary malignancies in females with primary fallopian tube cancer. International journal of cancer Journal international du cancer 120, 2047-2051.

Shih, H.A., Nathanson, K.L., Seal, S., Collins, N., Stratton, M.R., Rebbeck, T.R., and Weber, B.L. (2000). BRCA1 and BRCA2 mutations in breast cancer families with multiple primary cancers. Clinical cancer research : an official journal of the American Association for Cancer Research 6, 4259-4264.

Teppo, L., Pukkala, E., and Saxen, E. (1985). Multiple cancer--an epidemiologic exercise in Finland. Journal of the National Cancer Institute 75, 207-217.

Thompson, D., Easton, D.F., and Breast Cancer Linkage, C. (2002). Cancer Incidence in BRCA1 mutation carriers. Journal of the National Cancer Institute 94, 1358-1365.

van Asperen CJ, B.R., ; Meijers-Heijboer E J,; Hoogerbrugge N,; Verhoef S,; Vasen H,;, Ausems M, M.F., ; Gomez Garcia E,; Klijn J,; Hogervorst F,; van, Houwelingen J, v.t.V.L., ; Rookus M,; van Leeuwen F,; on behalf of the

Appendix cont.

Netherlands, and (HEBON), C.G.o.H.B.C. (2005). Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. J Med genet 42, 711-718.

Wagner, J.E., Tolar, J., Levran, O., Scholl, T., Deffenbaugh, A., Satagopan, J., Ben-Porat, L., Mah, K., Batish, S.D., Kutler, D.I., et al. (2004). Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia. Blood 103, 3226-3229.