

Evidence That *BRCA1*- or *BRCA2*-Associated Cancers Are Not Inevitable

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Inheriting a *BRCA1* or *BRCA2* gene mutation can cause a deficiency in repairing complex DNA damage. This step leads to genomic instability and probably contributes to an inherited predisposition to breast and ovarian cancer. Complex DNA damage has been viewed as an integral part of DNA replication before cell division. It causes temporary replication blocks, replication fork collapse, chromosome breaks and sister chromatid exchanges (SCEs). Chemical modification of DNA may also occur spontaneously as a byproduct of normal processes. Pathways containing *BRCA1* and *BRCA2* gene products are essential to repair spontaneous complex DNA damage or to carry out SCEs if repair is not possible. This scenario creates a theoretical limit that effectively means there are spontaneous *BRCA1/2*-associated cancers that cannot be prevented or delayed. However, much evidence for high rates of spontaneous DNA mutation is based on measuring SCEs by using bromodeoxyuridine (BrdU). Here we find that the routine use of BrdU has probably led to overestimating spontaneous DNA damage and SCEs because BrdU is itself a mutagen. Evidence based on spontaneous chromosome abnormalities and epidemiologic data indicates strong effects from exogenous mutagens and does not support the inevitability of cancer in all *BRCA1/2* mutation carriers. We therefore remove a theoretical argument that has limited efforts to develop chemoprevention strategies to delay or prevent cancers in *BRCA1/2* mutation carriers.

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INTRODUCTION

Inheriting a *BRCA1* or *BRCA2* gene mutation can cause a dual deficiency both in repairing some complex DNA damage and in forming sister chromatid exchanges (SCEs) for damage that cannot be repaired. Thus, any source of ongoing complex DNA damage places demands for normal *BRCA1/2* gene products. This scenario creates a theoretical limit that effectively means *BRCA1/2*-associated cancers occur spontaneously and cannot be prevented or delayed.

Some DNA lesions have been viewed as an integral part of DNA replication before cell division (1), and they cause temporary replication blocks and replication

fork collapse. This kind of replication stress can particularly affect genomic loci such as fragile sites, telomeres and repetitive sequences (2). DNA integrity is especially threatened if spontaneous reactions occur during the process of copying DNA before mitosis. During DNA copying, DNA polymerases stall at noncoding sites of DNA damage, and the replication fork may collapse. Stalled and collapsed replication forks can lead to chromosome breaks and micronucleus formation. They can also trigger SCEs (3). SCEs are widely believed to be a cytological manifestation of the repair of damaged or collapsed replication forks that occur all the time during DNA replication (4).

At the molecular level, SCE and homologous recombination are very similar. Identical (SCE) or similar (homologous recombination) segments of DNA line up and then cross over. SCEs do not necessarily lead to adverse health outcomes, but rather indicate complicated DNA damage that can be produced by many mutagens.

The persistence of lesions involved in SCEs for several cell divisions implies that, if lesions are not eliminated, SCEs represent a mechanism to tolerate them, allowing damaged cells to more safely replicate. The lesions may eventually be eliminated by repairs that take place during or after replication. SCEs and chromosomal breaks often occur in the same chromosomal regions (5). A high rate of spontaneous SCEs, even in normal individuals, has been used as major evidence for DNA damage during replication. Cancers would then become an almost inevitable consequence of cell division if *BRCA1/2*-mediated pathways are crippled.

However, much of the evidence for a high rate of spontaneous SCEs and DNA

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mutation is based on measurements made with bromodeoxyuridine (BrdU). BrdU is incorporated into newly synthesized DNA in dividing cells instead of deoxythymidine. Differential staining of BrdU-modified DNA allows the newly synthesized chromatid to be recognized because BrdU incorporation causes much weaker staining (6). When pieces of a BrdU chromatid exchange with its sister (which does not have BrdU), SCEs are directly visualized and can be counted.

SCE assays only measure sister chromatid crossover events, but do not measure noncrossover recombination. The frequency of noncrossover events is not necessarily tethered to that of crossover events. Thus, a low SCE rate is not necessarily sufficient to establish low mutagenic potential. However, only four mutations were found by direct sequencing of the human Y chromosome after 13 generations, and these were only single base substitutions (7). This produces an estimate of about 100 mutations over all human chromosomes per generation. Moreover, human DNA has remained stable because the human genome still shares >96% homology with chimpanzees, despite an accelerated mutation rate of the Y chromosome and the 5–7 million years since divergence (8,9). The estimate from direct Y-chromosome sequencing is consistent with estimates from published human chimpanzee comparisons for the same Y-chromosomal region and with other estimates of the rates of human mutation. Mutations that would alter the structure of some proteins such as fibrinopeptides occur once in an estimated 200,000 years.

Other Repairs Are Normal or Alternatives Exist

Reactive oxygen and nitrogen species are spontaneous mutagens that can affect DNA. In the 1990s, the use of inappropriate methods led to an overestimate of DNA oxidation products by up to three orders of magnitude (10). There are dedicated enzymes to repair some of this damage. For example, a DNA glycosylase prevents mutations associated with

8-oxoguanine, a common product of oxidative DNA damage (11), and this enzyme participates in base excision repair. 8-Oxoguanine is usually repaired before DNA replication and may not contribute to carcinogenesis. Other repair pathways such as nonhomologous end joining are still normal and are unaffected by the *BRCA1/2* mutation. In *BRCA1/2* mutation carriers, response even to some potent mutagens is still normal (12,13). Alternative less precise repairs may suffice, even if a normal *BRCA1/2*-mediated recombination pathway is crippled. Cells that have not lost the recombination process may have a growth advantage over cells with biallelic mutations. This step minimizes the effect of *BRCA1/2* deficits in permitting spontaneous mutations.

Other protective mechanisms are still normal. These mechanisms include checkpoint pathways and steric protection because new DNA is rapidly assembled onto nucleosomes, where it is supercoiled and tightly wound. After entry into mitosis, some fraction of chromosome regions with errors in DNA replication becomes sequestered into nuclear compartments containing p53 binding protein 1. These 53BP1 nuclear bodies shield DNA regions with replication problems against erosion and transmit them to future cell generations (2).

Finally, the whole organism has safeguards missing from cell culture. These include an intact immune system, detoxification systems and hazard avoidance. Although *Brca1* null strains can be generated in the mouse, homozygous loss of *BRCA1* is incompatible with human life. In these artificial mouse strains, mammary tumors appear to be spontaneous, but even low doses of ionizing radiation cause a marked increase in tumor formation (14). The mice also have increased tumors within the digestive tract on exposure to oxidative carcinogens. Thus, the homozygous mouse *Brca1* mutation increases risks from carcinogenic exposures. This raises the question whether some seemingly spontaneous tumors are actually related to ionizing radiation or other environmen-

tal carcinogens and might therefore be preventable.

Study Rationale

The present study was designed to test the data underlying the idea that cancer risk in *BRCA1/2* mutation carriers is so tightly linked to DNA replication that, every time cells in *BRCA1/2* mutation carriers divide, they accumulate chromosome aberrations. If this is true, exogenous genotoxins are unnecessary, and cancer becomes an unavoidable consequence of DNA replication (15).

Here, evidence is summarized that shows that the extent of spontaneous DNA damage manifested by SCEs has probably been overestimated. This finding brings SCE measurements closer to other measures of spontaneous DNA damage in mutation carriers. Measurement of DNA mutation by direct DNA sequencing of multiple generations suggests that non-SCE-associated mutation events are rare as well. Finding that SCEs have been overestimated reduces the numbers of *BRCA1/2*-associated cancers that are predicted as an inevitable consequence of cell replication. In contrast, the response to exogenous mutagens and carcinogens is clearly affected by mutation within pathways that require *BRCA1* and *BRCA2*. This evidence removes a theoretical argument that has limited efforts to develop chemoprevention strategies to delay or prevent cancers in *BRCA1/2* mutation carriers. There may be safe and feasible methods to control or compensate for DNA damage that triggers chromosome damage or chromatid exchanges in nonmutation carriers. Data supporting chemoprevention and other interventions in *BRCA1/2* mutation carriers may already exist but should still be considered as hypothetical. Considerably more investigation is needed before adopting patient care recommendations.

MATERIALS AND METHODS

Homologous recombination often uses the nascent sister chromatid to repair potentially lethal DNA lesions accompany-

ing replication (3). *BRCA1/2* genes encode for proteins required for repairs that suppress some SCEs, and *BRCA1/2* proteins are also required for these crossover events (16). Numbers of SCEs in the absence of added mutagens were therefore used to measure the overall requirements for *BRCA1/2* proteins caused by endogenous DNA damage. Searches for "BRCA1 and SCE" produced only five references. Searches were expanded to include ATM or Fanconi proteins and SCE. This is justified because ATM and Fanconi gene (*FA*) products participate with *BRCA1/2* proteins in a common pathway. PubMed literature references for all available years were searched for the words "sister chromatid exchanges" with the addition of words such as "*in vivo*," "nutrition," "diet," "spontaneous" and "antioxidant." The literature was scanned for effects of protectants and dietary intervention on the rates of SCEs. Key words included "SCE induction," "SCE diet," "SCE reduction" and "SCE chemicals." Because SCEs commonly occur at fragile sites, similar searches were conducted through the fragile site literature. Many studies made reference to chromosome aberrations and micronuclei, and these specific terms were used in additional searches.

Statistical Analysis

Testing of mean values representing varying numbers of samples was done by calculating two-sample *t* values (17,18) for comparisons of means from unpaired data. This assumes continuous distributions not too far from bell-shaped curves. Comparisons of mean values in the presence of mutagen versus control values were conducted by dozens of multiple two-sample *t* tests. Statistical significance data in the original article were included for articles reporting protection against SCE accompanying mutagen exposure. SCE control numbers versus SCEs in the presence of mutagens in studies of one to three individuals were compared by two-sample *t* testing of weighted averages and by the summary method of metaanalysis. Statistical calcu-

Table 1. Rates of spontaneous SCE determined by BrdU in normal human lymphocyte cultures differ among laboratories but increase greatly in response to exogenous mutagens.

Reference	Spontaneous SCEs \pm SD per cell determined by BrdU; n	Test mutagen	SCEs \pm SD (max)/cell with mutagen; n	Two-sample unpaired <i>t</i> test results, mutagen SCEs versus spontaneous SCEs
43	7.01 \pm 1.24; 22	Chemotherapy	11.52 \pm 3.33; 24	<i>P</i> < 0.001
44	5.175 \pm 0.607; 31	Chlormethine hydrochloride ^a	18.433 \pm 4.523; 10	<i>P</i> < 0.001
45	8.4 \pm 0.11; 5	Ptaquiloside	26.1 \pm 0.14; 5	<i>P</i> < 0.001
46	2.54 \pm 0.82; 20	Type 1 diabetes	5.44 \pm 1.47; 35	<i>P</i> < 0.001
47	21.84 \pm 4.21; 20	White fluorescent light in S phase	28.37 \pm 5.98	<i>P</i> = 0.0003
48	6.9 \pm 0.9; 22	Smoking	8.4 \pm 1.2; 18	NS
49	7.16 \pm 1.13; 15	Smoking	8.65 \pm 1.43; 14	NS
50	9.32 \pm 1.0; 8	Smoking	10.76 \pm 1.36; 6	NS
51	4.70 \pm 0.24; 2 4.48 \pm 0.46; 2	MMC ^b	34.82 \pm 1.25; 2	<i>P</i> < 0.0001 for weighted averages of SCEs from MMC versus SCEs in control cells (51–53)
52	4.45 \pm 0.68; 4	MMC ^b	53.37 \pm 5.1; 4	
53	5.95 \pm 0.31; 1 6.40 \pm 0.59; 1 6.64 \pm 0.47; 1	MMC	25.95 \pm 0.81; 1 23.52 \pm 1.30; 1	

n, Number of subjects; NS, not statistically significant.

^aAdded to cells as a positive control mutagen.

^bMMC greatly increased the frequency of chromosomal aberrations as independent supporting evidence.

lations were done by using the StatsDirect statistical program and with Microsoft Excel. The summary method of metaanalysis was done with StatsDirect.

RESULTS

To test whether *BRCA1/2*-related cancers are an unavoidable consequence of cell replication, numbers of spontaneous SCEs were compiled from the literature to estimate the normal rate of ongoing DNA recombination. Reports in Table 1 all used BrdU to measure background SCE. The first eight rows in Table 1 are references that reported mean values for spontaneous SCEs. Comparing these eight mean values by two-sample *t* tests (17) showed that most of the values have statistically significant differences from each other. There are 28 possible two-sample *t* test comparisons of spontaneous SCE levels in the eight references. Of these 28 two-sample *t* test compar-

isons, 24 showed statistically significant differences, no matter if the variances were assumed to be equal or unequal (two-tailed *P* < 0.05, with most <0.0001, all at high power).

Very large increases in SCEs versus controls can occur in the presence of exogenous mutagens (Table 1). Mutagens listed in the first five rows of Table 1 give greater numbers of SCEs than the spontaneous SCE values in the same row, and the differences are statistically significant (Table 1, last column). In contrast, the differences in SCEs associated with smoking versus controls (Table 1) are not statistically significant, with low power to detect differences. However, Table 1 does not consider differences in numbers of cells with high frequencies of SCEs. Data in Table 5 (see below) also refer to smoking, and Shim *et al.* (19) did find a statistically significant difference (*P* < 0.01) with high power to detect differences.

Table 2. Spontaneous SCEs measured in different systems or by different methods differ greatly from SCEs measured in human lymphocytes.

System and reference	Assay	Spontaneous SCE frequency	Test mutagen	SCE (max)/cell with mutagen	Independent supporting data
CHO cells in culture (54)	BrdU extrapolated to low doses	1.32/cell/cell cycle			Lower SCE frequency <i>in vivo</i>
Bovine autosomes 16 and 26 and Y chromosomes (55)	BrdU for one replication then CO-FISH	3.7/metaphase			
Ear fibroblasts from FYDR mice (20)	Fluorescent phenotype generated by homologous recombination event on chromosome 1	1.2-1.4 recombinants per million cell divisions	MMC	~150 recombinant cells/million cell divisions	
FYDR mice carrying a transgene; spontaneous HR in pancreas that contains few S phase cells (23)	Fluorescent phenotype representing homologous recombination event	2.5 recombinant cells/million cell divisions; some mice have zero recombinants; ~10/million cells in pancreas of individual animals (range ~0-300)	MMC	~90-135 per million cell divisions	p53 status does not affect spontaneous SCE frequency
Intact mouse embryo (21)	Expression of acetylcholine receptor due to recombination	1-2 × 10 ⁻⁶ /cell division			Frequency of recombination in intact embryo is similar to that in cultured cells
Human fibroblasts from a Lesch-Nyhan donor (56)	Growth in HAT medium due to recombination at HPRT locus	10-30 × 10 ⁻⁶ per cell per generation	Methyl nitrosoguanidine, UV, chromium oxide, MMC, DES, nickel chloride, sodium azide	Dose-dependent 2.4 -to 10-fold increase	Noncarcinogens showed much less activity Dose-dependent induction of HAT reversion is concordant with <i>in vivo</i> carcinogenesis

CHO, Chinese hamster ovary; CO-FISH, chromosome orientation fluorescence *in situ* hybridization; FYDR, fluorescent yellow direct repeat; HR, homologous recombination; HAT, hypoxanthine, amethopterin (methotrexate) and thymidine; UV, ultraviolet irradiation; HPRT, hypoxanthine-guanine phosphoribosyltransferase; DES, diethylstilbestrol.

Mitomycin C (MMC) is a mutagen and DNA cross-linking agent that requires pathways containing BRCA1/2 and Fanconi proteins for repairs. In counting SCEs by BrdU methods, MMC causes large increases (Table 1, last three rows). Doses of MMC that cause between 33% and 90% reduction in relative growth, increase SCEs about 100-fold (20), provided the cells remain viable. The last three rows in Table 1 (references 51-53) were based on measurements from one to four individuals. Summary metaanalysis of

these isolated data points gives a combined value of 5.39 (confidence interval [CI] 4.68-6.19) with an inconsistency statistic of 95.2% (highly inconsistent). Meta-analysis of MMC-associated SCEs gave a much higher summary value of 32.53 (25.34-41.75) but again with a very high inconsistency value (99.2%). There is no overlap in the CIs for the two groups, supporting the idea that the difference is statistically significant.

To further explore the role of chance in explaining differences attributed to

MMC, the weighted arithmetic mean of the spontaneous SCE values (Table 1, bottom three rows) was calculated as 5.30 ± 0.15. The weighted mean of the number of SCEs in the presence of MMC was calculated as 29.51 ± 0.58. This increase in SCEs due to the presence of MMC was statistically significant at a high power (>99%). A two-sample *t* test of spontaneous versus MMC weighted average values for SCEs gave *P* < 0.0001, whether or not variances were assumed to be equal.

Table 3. Carriers of mutations affecting pathways mediated by BRCA1/2 in humans have low levels of spontaneous chromatid aberrations, micronuclei and spontaneous SCEs.

Reference	System	Spontaneous chromatid exchanges, breaks or aberrations	Spontaneous SCEs as measured by BrdU MMC versus spontaneous
25	Fanconi anemia patient whole blood T-lymphocyte cultures	54% of homozygous Fanconi anemia patients do not have chromosome breakage	
57	Fanconi anemia lymphocytes and fibroblasts	Normal	Fivefold increase in the presence of an alkylating agent; fibroblasts were more sensitive than lymphocytes
58	<i>BRCA1</i> breast cancer families	5 of 9 <i>BRCA1</i> mutation carriers had 0 spontaneous chromosome aberrations, including 2/3 carriers in the same family; carriers with aberrations had them in only 2% of cells	
30	Peripheral blood lymphocytes from breast cancer patients with <i>BRCA1</i> or <i>BRCA2</i> mutation	No significant differences in mean spontaneous chromatid breaks or mean spontaneous micronuclei were observed among <i>BRCA1/2</i> patients, relatives and controls	
29	Human <i>BRCA1</i> -associated breast cancer	No increase in numerical chromosomal instability compared with sporadic tumors	
59	Human wild-type versus human homozygous <i>BCA2/FA-D1</i> and <i>FA-A B</i> lymphoblastoid cell lines and primary fibroblasts	Two Fanconi lymphoblastoid cell lines have only ~1-2 × the number of spontaneous chromatid breaks as wild-type; a third had over 5× normal; <i>FA-A</i> fibroblasts show about 2× greater spontaneous chromatid aberrations; <i>BRCA2/FA-D1</i> fibroblasts have 4.6 and 7.5× more	Vast majority of MMC-treated cells die but SCE numbers in surviving cells are ~1-2× spontaneous

FA, Fanconi anemia.

Most studies cited in Table 1 provided independent evidence supporting the idea that exogenous mutagens (rather than chance) are associated with increased SCE levels. This evidence includes comet assay results that are independent of BrdU, dose-response relationships, greatly increased chromosome aberrations, concordance with carcinogenicity under limited specialized conditions and the absence of SCEs with nonmutagens.

In Table 2, extrapolation of BrdU concentrations to low doses shows just over one SCE per cell division (Table 2, first row). *In vivo* methods generally give lower rates of SCE than *in vitro* methods (Table 2). In contrast to using BrdU to measure overall spontaneous recombination, spe-

cialized methods that mark a specific DNA region give results that are millions of times less than the low dose value of one SCE per cell division and the values in Table 1. These results yield about one or only a few recombinants per million cell divisions. By use of an artificial gene transferred to mice that fluoresces yellow on recombination, recombination rates of about 1 per million cell divisions were obtained. Spontaneous recombination rates of between 1 and 2×10^{-6} were also measured using tandem repeat sequences (21). Many juvenile mice have zero recombinants, and there is a wide range of recombinant cell frequencies (22) (Table 2).

Spontaneous recombination levels of $15.6 (\pm 1.6)$ per million cells are threefold

lower than the background levels observed for immortalized human cells at the same duplication (23). Either value is far below widely cited rates of spontaneous recombination. Background SCEs and homologous recombination are lower in normal cells, which undergo homologous recombination much less frequently than immortal cell lines.

There is only limited data related to SCEs in *BRCA1/2* mutation carriers, but some studies have measured chromosome damage or SCEs in human cells with defects in other genes related to *BRCA1/2* functions. Fanconi anemia is a rare syndrome characterized by bone marrow failure, malformations and cancer predisposition. In Fanconi anemia,

Table 4. Rates of cancer in mutation-negative relatives in *BRCA1/2* families.

Reference	Number of patients mutation negative	Relationship to mutation carrier	Length of follow-up	Breast and other cancer risk (CI) and comments
33	1,492	FDR	Mean 6.1 years, followed up to age 75	Standardized incidence ratio (SIR) 4.3 Ovarian and colon cancer risks were slightly below normal
60	104	FDR/SDR	Median 8 years	SIR 2.9 (1.0–8.6)
61	11,378	FDR	NA	Relative risk (RR) 0.39 (0.04–3.8); substantial risk heterogeneity High-risk women account for only 3.4% of the female populations in three countries studied, but 32% of all breast cancer cases; uses a constant value for risk of carriers; assumes one additional latent gene to account for all risk
62	184	FDR	NA	SIR 5.0
63	395	FDR/SDR/TDR	Entry at 31.3 years followed for average of 17.7 years to age 48; third-degree relative to age 41	For FDR, SIR 1.33 (0.49–2.91); all RR = 0.75 (0.34–1.41); assumptions about oophorectomy
64	130.5	FDR	Diagnosis before age 50	Odds ratio (OR) 2
65	442	FDR/SDR	Median 6.1 years	SIR 1.29 (0.58–2.88); excluded 400 women with a prior cancer diagnosis
66	375	FDR/SDR mostly	Median age 44 mean follow up 4.9 years	SIR 2.3 (0.57–9.19) for <i>in situ</i> breast cancer
67	3,742	FDR	NA	OR 1.6 (1.2–2.1)

FDR/SDR/TDR, first-/second-/third-degree relatives; NA, not specified.

one of the genes encoding a Fanconi protein has an inherited homozygous or biallelic mutation. Both the genomic deficit and the cancer risks are more serious than a heterozygous *BRCA1/2* mutation.

Fanconi anemia has long been considered a spontaneous chromosome fragility syndrome. Leukemia is common but the age of onset of leukemia is variable (24). On average, the Fanconi anemia population has an increased level of spontaneous DNA damage, but 54% of patients with Fanconi anemia (excluding mosaics) have a spontaneous chromosome fragility level within the range of normal patients (25). Therefore, spontaneous chromosome fragility cannot be used as a diagnostic tool for Fanconi anemia. For Fanconi anemia G, background levels of chromatid interchanges and other aberrations in Fanconi anemia are also close to those of normal cells (26) (Table 3).

Widely available and circulated photos of human Fanconi chromosomes are all in the presence of MMC. Chromosome fragility induced by DNA inter-strand cross-link-inducing agents such as MMC is the gold standard test to diagnose Fanconi anemia. Encoded products of Fanconi (*FA*) genes all participate in a common pathway with *BRCA1* and *BRCA2* to repair MMC-induced cross-links, double strand breaks and complex DNA damage. MMC in *BRCA2* (*FA-D1*) null cells causes chromatid aberrations such as interchanges, intrachanges, triradials or subchromatid exchanges, representing aberrant repairs and/or chemical cross-linking. Many of the breaks occur at sites that grossly appear to be the same places where SCEs occur (5).

Experiments with exogenous mutagens such as MMC, however, are hampered by essentially zero survival of

Brca2-deficient cells because of MMC (27). Only minimally damaged cells reach the second metaphase in the presence of MMC (5). Because MMC kills almost all cells in some experiments, SCE rates are found to be similar to background levels and may only reflect cells that were not severely damaged. This explains why BrdU can give similar or even lower SCE levels for spontaneous and mutagen-treated Fanconi cells.

There are serious problems in measuring spontaneous and induced chromosome aberrations in cell lines that have a homozygous or biallelic Fanconi mutation. The source of these problems may be related to a low cloning efficiency, damage from cell culture, unidentified mutagen exposures, variation in other genes and, in some cases, the severity of the Fanconi mutation. Despite these complications, numbers of chromosome aber-

Table 5. Common mutagens that increase SCE and protective factors that reduce SCEs measured with BrdU.

SCE control/cell	System tested with BrdU and reference	Test protective agent	Mutagen and SCE with mutagen	SCE with protectant	Statistical test of significance
Nonsmokers 7.03 ± 0.33 (n = 9); smokers (9.46 ± 0.46) (n = 14)	Blood specimens from clinically healthy male subjects between 20 and 52 years of age (19)	Green tea and plant polyphenols	Cigarette smokers, 9.46 ± 0.46	Smoker SCEs (7.94 ± 0.31) were comparable to those of nonsmokers (7.03 ± 0.33)	Smoking cigarettes and drinking green tea significantly affected SCE frequency and explained 32.7% of SCE variation ($P < 0.002$) (19)
3.36 ± 0.24	Mouse bone marrow cells <i>in vivo</i> (68)	Green tea extract given to anesthetized animals	Dimethyl-nitrosamine 23.40 ± 0.94 at 18 h	12.12 ± 0.07 (a similar effect at 24 h; more modest effects at other times)	The suppression rate was significantly different from mice given only dimethyl nitrosamine (t test $P < 0.05$) (68)
4.1 ± 0.46 for not fasted (29% cells have 0–2 SCEs)	Male Wistar rats every other day fasting 12 wks (69)	Dietary limitations	Diet	1.8 ± 0.12 for fasted (72% cells have 0–2 SCEs)	Significant differences in the numbers of SCEs (t test $P < 0.05$) (69)
4 at 6 h to 6 at 48 h exposure to radiation	Human peripheral blood lymphocytes exposed to 1.8 Ghz radiation (70)	Ginkgo biloba	RF/microwave radiation 8 at 6 h to 13 at 48 h	6 at 6 h and about 7 at 48 h	There was a significant increase ($P < 0.05$) in SCE frequency in RF-exposed lymphocytes compared with sham controls (70)
0.24 ± 0.12/cell	Traffic policemen in Bangkok Thailand (71)		4.40 ± 0.93/cell with Benzene, toluene, CO, formaldehyde, etc.		A significantly higher SCE frequency in policemen was observed ($P < 0.05$) (71)
4.3 ± 0.19	Human lymphocytes (72)		6.64 ± 0.88 with hydroquinone, a product of benzene metabolism		Hydroquinone significantly increased micronuclei and SCE ($P < 0.0001$) (72)
4.6 ± 0.37 to 6.0 ± 0.39/cell	CHO-K1 cells (73)	Ascorbate, glutathione	8.6 ± 0.52 Phenyl hydroquinone, a metabolite of o-phenylphenol, an agricultural fungicide and surface disinfectant (Lysol)	5.1 ± 0.39 with ascorbate, 4.6 ± 0.37 with glutathione	Ascorbate and glutathione significantly decrease SCEs versus Phenyl hydroquinone alone ($P < 0.05$) (73)
12/cell	Ataxia telangiectasia patient cells (affects BRCA1/2-mediated pathways) (4)		18 after 2 Gy radiation		

Continued

rations in Fanconi cell lines can still show significant overlap with normal cells (25). Two Fanconi lymphoblastoid

cell lines had only about one to two times the number of spontaneous chromatid breaks as the wild-type cell line.

Fibroblast cell lines derived from Fanconi anemia type A patients show about two times greater spontaneous chromatid

Table 5. Continued

5.2 ± 1.53/cell	Human peripheral blood lymphocytes (74)	Broccoli head extract	MMC 72.7 ± 8.57	33.33 ± 1.71	Results with mutagen showed significant differences from untreated control and significant differences between mutagen and mutagen plus broccoli extract ($P < 0.05$) (74)
0.18 ± 0.01/cell	Chinese hamster fibroblasts (75)	Squalene	H ₂ O ₂ 0.39 ± 0.01	0.24 ± 0.05	Pretreatment with squalene significantly decreased the frequency of SCE induced by H ₂ O ₂ ($P < 0.05$)
0.244 ± 0.003/cell	Chinese hamster V79 cells (76)	α-Tocopherol	H ₂ O ₂ 0.49 ± 0.01	0.252 ± 0.003	Increased SCEs and protection were statistically significant ($P < 0.05$)
All cells from niacin-deficient rats had ≥5 SCEs; 51.2% had 5–10; 48.8% had ≥11 SCEs	Rat bone marrow cells (77) IP BrdU injection <i>in vivo</i> 1 mg/g	Niacin	Etoposide 28.4% of niacin-deficient rats had 5–10 SCEs and 71.6% had ≥11	83% of control cells had 0–4 SCEs; 17% had 5–10 SCEs	

RF, radio frequency; IP, intraperitoneal.

aberrations. However there are higher levels in other cell lines (25,27) (Table 3).

Evidence That Spontaneous Rates of DNA Damage Are Low in Mutation Carriers

Data in Table 2 suggest there are actually only small numbers of spontaneous SCEs. Table 3 shows that chromatid aberrations and micronuclei are often near normal, even for severe deficits. *BRCA1* and *BRCA2* carriers with or without breast cancer do not exhibit a marked difference in chromosome stability relative to controls (28,29). However, damage from MMC is greatly amplified in mutation carriers to the point of cell death. About half the *in vivo* and *in vitro* studies find a mutation-associated increase in chromosome damage from radiation (30). Recent epidemiologic data provide further evidence that even low-level radiation doses (such as from chest X-rays) in-

crease cancer risks in *BRCA1/2* mutation carriers (31,32).

Data in Table 3 therefore further implicate environmental factors or other gene variants in increasing cancer incidence in *BRCA1/2* families. To test this idea, we next determined whether such modifiers have been detected in epidemiological studies. Table 4 presents cancer risks in members of *BRCA* families who did not have the familial mutation. Although most studies in Table 4 suggest an increase in risk in *BRCA1/2* family members who do not have the familial mutation, confidence intervals often include one. One study did find statistically significant breast cancer risk was higher for women between the ages of 50 and 70 years (1% per year) than for women between the ages of 30 and 50 years (0.4% per year) (33). Yet other studies in Table 4 did not follow family members past age 50 years. Close relatives of patients with breast can-

cer are more likely than other women to belong to a high-risk breast cancer group, even if they do not have the familial *BRCA* mutation. Inconsistency in results among the studies suggests additional variables. These could include environmental differences and variation in other genes, which may also be sensitive to environment or diet. A large population-based, case-control study (34) found broad variation in breast cancer risk among carriers of *BRCA1* and *BRCA2* mutations. There was no single risk associated with having a *BRCA1/2* mutation.

Healthy Carrier and Noncarriers in *BRCA* Families Show About the Same Amounts of DNA Damage

Changing the environment to one with lower cancer rates may lower the incidence of breast cancer in mutation carriers. Ashkenazi Jewish women are roughly 10 times more likely to be

BRCA1/2 mutation carriers than other women; therefore, Ashkenazi Jewish women have high breast cancer risks as a group. In contrast, the breast cancer risk of the Brazilian population is much lower (roughly half the rate of the U.S. population). Ashkenazi Jewish women who move to Sao Paulo or Porto Alegre, Brazil, reduce their breast cancer mortality much closer to the lower breast cancer mortality of the general Brazilian population at all ages except age >79 years. Age-adjusted breast cancer mortality rates were 24.1/100,000 (CI 13.5–39.7) among Ashkenazi women in the two cities in Brazil and 22.3/100,000 women in the general population. These data support the influence of the environment on breast cancer risk. Even a high-risk population can reduce their cancer risks (35).

Chemoprotection. Despite its overestimate of spontaneous endogenous DNA damage and its other potential limitations, BrdU has been used to compare effectiveness of protection against mutagen-related exogenous damage. The effects of these or other mutagens such as viruses cannot be fully explained by increased oxidative stress. Mutagens listed in Table 5 include known carcinogens, and exposure to them can probably be reduced or avoided. Green tea, dietary limitation, ascorbate, glutathione, broccoli and correction of niacin deficiency have been suggested as capable of preventing the increase in SCE levels (Table 5). Most articles in Table 5 verified the statistical significance of their findings (Table 5, last column). Table 5 therefore shows that mutagens increase the numbers of SCE and that there may be specific pharmacologic agents that can prevent some of this increase.

DISCUSSION

We hope that this work will be a prelude to a much-needed discussion of the inevitability of hereditary cancers. There is a popular assumption that high levels of DNA cross-linking, double strand breaks and their resolution are going on all the time during cell replication. DNA is thought to be under further constant

attack from byproducts of normal ongoing processes. *BRCA1/2* mutation carriers have a dual deficit in repairing complex DNA damage and in forming SCEs. It follows that a mutation in *BRCA1* or in *BRCA2* genes means that cancers in mutation carriers are inevitable. Preventing cancers related to *BRCA1/2* deficiencies has not been viewed as realistic. The rate of normal spontaneous SCEs reflecting endogenous damage has been especially overestimated and depends on the method of measurement. A lowered rate of spontaneous SCEs agrees with low rates of spontaneous DNA mutation, direct DNA sequencing and measurements of spontaneous chromosome damage. Until recently, some oxidation products have also been overestimated, and experimental support for spontaneous oxidative cross-links is not strong. All these considerations reduce the requirements for *BRCA1/2*-mediated processes. Moreover, independent evidence shows that environmental and other genetic factors are also involved in determining risk. Risks are elevated for breast cancers in members of *BRCA1/2* families who do not have the familial mutation. Even mouse models with very severe *Brca1* deficits (14) show increased cancer risks from their environment. Changing the environment by moving to an area with lower cancer rates may reduce *BRCA* mutation-related breast cancer risks (35).

Other single gene diseases different from *BRCA1/2* deficiencies can be managed by pharmacology, by diet and/or by other interventions. Pharmacologic interventions in *BRCA1/2* mutation carriers have largely concentrated on better methods of destroying damaged cells or on blocking the estrogen receptor (36). The data in Table 5 imply that there are chemopreventive agents in foods or natural products that oppose the effects of some mutagens. Whether these interventions will reduce the cancer burden in *BRCA1/2* mutation carriers requires further testing.

Recent genomic data have reported that breast cancers are mutationally heterogeneous. There is substantial variation in so-

matic mutation and in indels. The ability to suppress mutagen-associated SCEs depends on *BRCA1/2* pathway activity. Genomic results are quite consistent with differential exposures and responses to mutagens as contributors to shaping cancer tissue distribution (37,38). Very little is known about what causes these mutations. Whether carcinogenicity of some mutagens is susceptible to chemoprevention requires further testing. Chemoprevention may be helpful whether the source is exogenous or endogenous.

A recent model on the basis of cancer DNA sequence results suggests clusters of DNA lesions precede large-scale genomic rearrangements. Sequences of *BRCA1*- and *BRCA2*-associated breast cancers show enrichment of somatic rearrangements and fusions in critical protein coding genes (39). Some mutagens such as radiation exposure and inflammation can encourage these kinds of rearrangements by causing extensive and complex DNA damage that collapses replication forks, causes double strand breaks or requires homologous recombination repairs. This increases requirements for *BRCA1/2* pathway activities, but the mutagens are exogenous.

Another source of mutation could be related to micronutrient deficits. Epidemiological studies reveal strong association between micronutrient deficiencies and development of cancer. A prevailing idea is that marginal micronutrient deficiencies lead to allocation of scarce cellular resources toward immediate survival at the expense of maintaining genomic integrity. This result places the individual at greater risk for degenerative diseases and cancer in old age (40).

BrdU-based methods have probably overestimated spontaneous SCEs because BrdU is itself a mutagen and a potent carcinogen that causes SCEs (41). DNA lesions caused by BrdU are difficult to avoid in replicating cells because BrdU enters the nucleotide pool and then becomes incorporated into DNA. BrdU induces transcription of silenced genes, disrupts nucleosome positioning by inducing A-form-like DNA conformation

in yeast cells, induces a senescent type phenotype, decondenses particular regions of chromosomes and alters specific chromatin structures (42).

A limitation of our results lies in the extent to which BrdU amplifies the effects of other mutagens. Higher levels of BrdU incorporation may increase the sensitivity of detection of both spontaneous and mutagen-related SCEs. However, in normal cells, exogenous increases in SCEs measured with BrdU are consistent with other systems of measurement, such as fluorescence detection of recombination and comet assays (Table 2). Dose response curves and extrapolation to low BrdU doses also support a large difference between SCEs caused by BrdU versus other mutagens.

CONCLUSION

Pathways containing *BRCA1* and *BRCA2* gene products are thought essential to repair spontaneous complex DNA damage or to carry out SCEs if repair is not possible. A high level of natural, unavoidable complex DNA damage represents a theoretical limit that would effectively mean most *BRCA1/2*-associated cancers are spontaneous and cannot be prevented or delayed.

Much evidence, however, for high rates of spontaneous DNA mutation is based on measuring SCEs using BrdU. Here we find that the routine use of BrdU has probably led to overestimating spontaneous DNA damage and SCEs because BrdU is itself a mutagen. Evidence based on spontaneous chromosome abnormalities and epidemiologic data indicate strong effects from exogenous mutagens and does not support the inevitability of cancer in all *BRCA1/2* mutation carriers. We therefore remove a theoretical argument that has limited efforts to develop chemoprevention strategies to delay or prevent cancers in *BRCA1/2* mutation carriers.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

- Kowalczykowski SC. (2000) Initiation of genetic recombination and recombination-dependent replication. *Trends Biochem. Sci.* 25:156–65.
- Lukas C, et al. (2011) 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. *Nat. Cell Biol.* 13:243–53.
- Sonoda E, et al. (1999) Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells. *Mol. Cell. Biol.* 19:5166–9.
- White J, Choi S, Bakkenist C. (2012) Transient ATM kinase inhibition disrupts DNA damage-induced sister chromatid exchange. *Sci. Signal.* 3:ra44.
- Latt SA, Stetten G, Juergens LA, Buchanan GR, Gerald PS. (1975) Induction by alkylating agents of sister chromatid exchanges and chromatid breaks in Fanconi's anemia. *Proc. Natl. Acad. Sci. U. S. A.* 72:4066–70.
- Bayani J, Squire JA. (2005) Sister chromatid exchange. *Curr. Protoc. Cell Biol.* 22.7.1–4.
- Xue Y, et al. (2009) Human Y chromosome base-substitution mutation rate measured by direct sequencing in a deep-rooting pedigree. *Curr. Biol.* 19:1453–7.
- Kuroki Y, et al. (2006) Comparative analysis of chimpanzee and human Y chromosomes unveils complex evolutionary pathway. *Nat. Genet.* 38:158–67.
- Akopian V, et al. (2012) Epigenomics and chromatin dynamics. *Genome Biol.* 13:313.
- Cadet J, Ravanat JL, Tavernaporro M, Menoni H, Angelov D. (2012) Oxidatively generated complex DNA damage: tandem and clustered lesions. *Cancer Lett.* 327:5–15.
- David SS, O'Shea VL, Kundu S. (2007) Base-excision repair of oxidative DNA damage. *Nature.* 447:941–50.
- de Campos-Nebel M, Larripa I, Gonzalez-Cid M. (2008) Non-homologous end joining is the responsible pathway for the repair of fludarabine-induced DNA double strand breaks in mammalian cells. *Mutat. Res.* 646:8–16.
- Trenz K, Landgraf J, Speit G. (2003) Mutagen sensitivity of human lymphoblastoid cells with a *BRCA1* mutation. *Breast Cancer Res. Treat.* 78:69–79.
- Kim SS, et al. (2009) Impaired skin and mammary gland development and increased gamma-irradiation-induced tumorigenesis in mice carrying a mutation of S1152-ATM phosphorylation site in *Brca1*. *Cancer Res.* 69:9291–300.
- Venkitaraman AR. (2009) Linking the cellular functions of *BRCA* genes to cancer pathogenesis and treatment. *Ann. Rev. Pathol.* 4:461–87.
- Feng Z, Zhang J. (2012) A dual role of *BRCA1* in two distinct homologous recombination mediated repair in response to replication arrest. *Nucleic Acids Res.* 40:726–38.
- Whitley E, Ball J. (2002) Statistics review 5: comparison of means. *Crit. Care.* 6:424–8.
- Whitley E, Ball J. (2002) Statistics review 2: samples and populations. *Crit. Care.* 6:143–8.
- Shim JS, et al. (1995) Chemopreventive effect of green tea (*Camellia sinensis*) among cigarette smokers. *Cancer Epidemiol. Biomarkers Prev.* 4:387–91.
- Hendricks CA, et al. (2003) Spontaneous mitotic homologous recombination at an enhanced yellow fluorescent protein (EYFP) cDNA direct repeat in transgenic mice. *Proc. Natl. Acad. Sci. U. S. A.* 100:6325–30.
- Lambert S, et al. (1999) Analysis of intrachromosomal homologous recombination in mammalian cell, using tandem repeat sequences. *Mutat. Res.* 433:159–68.
- Wiktor-Brown DM, Hendricks CA, Olipitz W, Engelward BP. (2006) Age-dependent accumulation of recombinant cells in the mouse pancreas revealed by in situ fluorescence imaging. *Proc. Natl. Acad. Sci. U. S. A.* 103:11862–7.
- Wiktor-Brown DM, Sukup-Jackson MR, Fakhraldeen SA, Hendricks CA, Engelward BP. (2011) p53 null fluorescent yellow direct repeat (FYDR) mice have normal levels of homologous recombination. *DNA Repair.* 10:1294–9.
- Glanz A, Fraser FC. (1982) Spectrum of anomalies in Fanconi anaemia. *J. Med. Genet.* 19:412–6.
- Castella M, et al. (2011) Chromosome fragility in patients with Fanconi anaemia: diagnostic implications and clinical impact. *J. Med. Genet.* 48:242–50.
- Tebbs RS, et al. (2005) New insights into the Fanconi anemia pathway from an isogenic *FancG* hamster CHO mutant. *DNA Repair.* 4:11–22.
- Kraakman-van der Zwet M, et al. (2002) *Brca2* (*XRCC11*) deficiency results in radioresistant DNA synthesis and a higher frequency of spontaneous deletions. *Mol. Cell. Biol.* 22:669–79.
- Beetstra S, et al. (2006) Lymphocytes of *BRCA1* and *BRCA2* germ-line mutation carriers, with or without breast cancer, are not abnormally sensitive to the chromosome damaging effect of moderate folate deficiency. *Carcinogenesis.* 27:517–24.
- Focken T, et al. (2011) Human *BRCA1*-associated breast cancer: no increase in numerical chromosomal instability compared to sporadic tumors. *Cytogenet. Genome Res.* 135:84–92.
- Baeyens A, et al. (2004) Chromosomal radiosensitivity in *BRCA1* and *BRCA2* mutation carriers. *Int. J. Radiat. Biol.* 80:745–56.
- Andrieu N, et al. (2006) Effect of chest X-rays on the risk of breast cancer among *BRCA1/2* mutation carriers in the international *BRCA1/2* carrier cohort study: a report from the EMBRACE, GENEPSO, GEO-HEBON, and IBCCS Collaborators' Group. *J. Clin. Oncol.* 24:3361–6.
- Lecarpentier J, et al. (2011) Variation in breast cancer risk with mutation position, smoking, alcohol, and chest X-ray history, in the French

- National BRCA1/2 carrier cohort (GENEPSO). *Breast Cancer Res. Treat.* 130:927–38.
33. Metcalfe KA, et al. (2009) Breast cancer risks in women with a family history of breast or ovarian cancer who have tested negative for a BRCA1 or BRCA2 mutation. *Br. J. Cancer.* 100:421–5.
 34. Begg CB, et al. (2008) Variation of breast cancer risk among BRCA1/2 carriers. *JAMA.* 299:194–201.
 35. Koifman S, Jorge Koifman R. (2001) Breast cancer mortality among Ashkenazi Jewish women in Sao Paulo and Porto Alegre, Brazil. *Breast Cancer Res.* 3:270–5.
 36. King MC, et al. (2001) Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2: National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) Breast Cancer Prevention Trial. *JAMA.* 286:2251–6.
 37. Walter MJ, et al. (2012) Clonal architecture of secondary acute myeloid leukemia. *N. Engl J. Med.* 366:1090–8.
 38. Friedenson B. (2010) A theory that explains the tissue specificity of BRCA1/2 related and other hereditary cancers. *Journal of Medicine and Medical Sciences.* 1:372–84.
 39. Kanikarla-Marie P, Ronald S, De Benedetti A. (2011) Nucleosome resection at a double-strand break during non-homologous ends joining in mammalian cells: implications from repressive chromatin organization and the role of ARTEMIS. *BMC Research Notes* 4:13.
 40. Lal A, Ames BN. (2011) Association of chromosome damage detected as micronuclei with hematological diseases and micronutrient status. *Mutagenesis.* 26:57–62.
 41. Kanda R, Yamagishi Y, Hayata I. (2004) Sister chromatid exchanges in ring chromosomes following X-irradiation of human lymphocytes. *Int. J. Radiat. Biol.* 80:363–8.
 42. Miki K, et al. (2010) 5-Bromodeoxyuridine induces transcription of repressed genes with disruption of nucleosome positioning. *FEBS J.* 277:4539–48.
 43. Tekcan A, Elbistan M, Ulusoy AN. (2012) Sister chromatid exchanges in breast cancer patients who underwent chemotherapy. *J. Toxicol. Sci.* 37:235–43.
 44. Wiczak M, Kociszewska I, Wilczynski J, Lopaczynska D, Ferenc T. (2010) Evaluation of chromosome aberrations, sister chromatid exchange and micronuclei in cultured cord-blood lymphocytes of newborns of women treated for epilepsy during pregnancy. *Mutat. Res.* 701:111–7.
 45. Gil da Costa RM, et al. (2012) Multiple genotoxic activities of ptaquiloside in human lymphocytes: auegenesis, clastogenesis and induction of sister chromatid exchange. *Mutat. Res.* 747:77–81.
 46. Cinkilic N, et al. (2009) Evaluation of chromosome aberrations, sister chromatid exchange and micronuclei in patients with type-1 diabetes mellitus. *Mutat. Res.* 676:1–4.
 47. Amorim MI, et al. (2008) Genotoxic effects of white fluorescent light on human lymphocytes in vitro. *Mutat. Res.* 652:204–7.
 48. Cheng TJ, et al. (1995) Comparison of sister chromatid exchange frequency in peripheral lymphocytes in lung cancer cases and controls. *Mutat. Res.* 348:75–82.
 49. Ben Salah G, et al. (2011) Sister chromatid exchange (SCE) and high-frequency cells (HFC) in peripheral blood lymphocytes of healthy Tunisian smokers. *Mutat. Res.* 719:1–6.
 50. Tucker JD, Ashworth LK, Johnston GR, Allen NA, Carrano AV. (1988) Variation in the human lymphocyte sister-chromatid exchange frequency: results of a long-term longitudinal study. *Mutat. Res.* 204:435–44.
 51. Celik M, et al. (2006) Effects of Thymus kotschyanus var. glabrescens Boiss. extract on mitomycin-C induced chromosomal aberrations and sister chromatid exchanges in human lymphocytes. *Cytotechnology.* 51:99–104.
 52. Kaya FF, Topaktas M. (2007) Genotoxic effects of potassium bromate on human peripheral lymphocytes in vitro. *Mutat. Res.* 626:48–52.
 53. Vernole P, et al. (1988) Sister-chromatid exchanges in human lymphocytes exposed to 1-p-(3-methyltriazeno)benzoic acid potassium salt. *Mutat. Res.* 208:233–6.
 54. Pinero J, Daza P, Escalza P, Cortes F. (1992) Influence of low doses of BrdU and estimation of spontaneous SCE in CHO chromosomes: three-way differential staining and an immunoperoxidase method. *Chromosoma.* 102:66–70.
 55. Revay T, King WA. (2012) Sister chromatid exchange assessment by chromosome orientation-fluorescence in situ hybridization on the bovine sex chromosomes and autosomes 16 and 26. *Cytogenet Genome Res* 136:107–16.
 56. Li J, Ayyadevera R, Shmookler Reis RJ. (1997) Carcinogens stimulate intrachromosomal homologous recombination at an endogenous locus in human diploid fibroblasts. *Mutat. Res.* 385:173–93.
 57. Hayashi K, Schmid W. (1975) The rate of sister chromatid exchanges parallel to spontaneous chromosome breakage in Fanconi's anemia and to trenimon-induced aberrations in human lymphocytes and fibroblasts. *Humangenetik.* 29:201–6.
 58. Baria K, et al. (2001) Correspondence re: A. Rothfuss et al. Induced micronucleus frequencies in peripheral blood lymphocytes as a screening test for carriers of a BRCA1 mutation in breast cancer families. *Cancer Res.* 60:390–4, 2000. *Cancer Res.* 61:5948–9.
 59. Godthelp BC, et al. (2006) Cellular characterization of cells from the Fanconi anemia complementation group, FA-D1/BRCA2. *Mutat. Res.* 601:191–201.
 60. Rowan E, Poll A, Narod SA. (2007) A prospective study of breast cancer risk in relatives of BRCA1/BRCA2 mutation carriers. *J. Med. Genet.* 44:e89.
 61. Kurian AW, et al. (2011) Breast cancer risk for noncarriers of family-specific BRCA1 and BRCA2 mutations: findings from the Breast Cancer Family Registry. *J. Clin. Oncol.* 29:4505–9.
 62. Smith A, et al. (2007) Phenocopies in BRCA1 and BRCA2 families: evidence for modifier genes and implications for screening. *J. Med. Genet.* 44:10–5.
 63. Korde LA, et al. (2011) No evidence of excess breast cancer risk among mutation-negative women from BRCA mutation-positive families. *Breast Cancer Res. Treat.* 125:169–73.
 64. Gronwald J, Cybulski C, Lubinski J, Narod SA. (2007) Phenocopies in breast cancer 1 (BRCA1) families: implications for genetic counselling. *J. Med. Genet.* 44:e76.
 65. Harvey SL, et al. (2011) Prospective study of breast cancer risk for mutation negative women from BRCA1 or BRCA2 mutation positive families. *Breast Cancer Res. Treat.* 130:1057–61.
 66. Domchek SM, et al. (2010) Breast cancer risks in individuals testing negative for a known family mutation in BRCA1 or BRCA2. *Breast Cancer Res. Treat.* 119:409–14.
 67. Katki HA, Gail MH, Greene MH. (2007) Breast-cancer risk in BRCA-mutation-negative women from BRCA-mutation-positive families. *Lancet Oncol.* 8:1042–3.
 68. Al-Fify Z, Aly M. (2010) Protective effect of green tea against dimethylnitrosamine induced genotoxicity in mice bone marrow cells. *Open Cancer J.* 3:16–21.
 69. Azab M, Khabour OF, Al-Omari L, Alzubi MA, Alzoubi K. (2009) Effect of every-other-day fasting on spontaneous chromosomal damage in rat's bone-marrow cells. *J. Toxicol. Environ. Health A.* 72:295–300.
 70. Esmekaya MA, et al. (2011) Mutagenic and morphologic impacts of 1.8GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). *Sci. Total Environ.* 410–411:59–64.
 71. Soogarun S, Suwansakri J, Wiwanitkit V. (2006) High sister chromatid exchange among a sample of traffic policemen in Bangkok, Thailand. *Southeast Asian J. Trop. Med. Public Health.* 37:578–80.
 72. Silva Mdo C, Gaspar J, Duarte Silva I, Faber A, Rueff J. (2004) GSTM1, GSTT1, and GSTP1 genotypes and the genotoxicity of hydroquinone in human lymphocytes. *Environ. Mol. Mutagen.* 43:258–64.
 73. Tayama S, Nakagawa Y. (1994) Effect of scavengers of active oxygen species on cell damage caused in CHO-K1 cells by phenylhydroquinone, an o-phenylphenol metabolite. *Mutat. Res.* 324:121–31.
 74. Anupama M, Murgan SS, Murthy PB. (2008) Broccoli flower head extract reduces mitomycin-C induced sister chromatid exchange in cultured human lymphocytes. *Food Chem. Toxicol.* 46:3351–3.
 75. O'Sullivan L, Woods J, O'Brien N. (2002) Squalene but not n-3 fatty acids protect against hydrogen peroxide-induced sister chromatid exchanges in Chinese hamster V-79 cells. *Nutr. Res.* 22:847–58.
 76. O'Leary KA, Woods JA, O'Brien NM. (2001) Gamma-tocopherol is less effective than alpha-tocopherol in preventing oxidant-induced sister chromatid exchanges in Chinese hamster V79 cells. *Free Radic. Res.* 35:917–24.
 77. Spronck JC, Kirkland JB. (2002) Niacin deficiency increases spontaneous and etoposide-induced chromosomal instability in rat bone marrow cells in vivo. *Mutat. Res.* 508:83–97.