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ARTICLE

Elevated Levels of Somatic Mutation in a Manifesting BRCA1 Mutation Carrier

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Homozygous loss of activity at the breast cancer-predisposing genes *BRCA1* and *BRCA2* (*FANCD1*) confers increased susceptibility to DNA double strand breaks, but this genotype occurs only in the tumor itself, following loss of heterozygosity at one of these loci. Thus, if these genes play a role in tumor etiology as opposed to tumor progression, they must manifest a heterozygous phenotype at the cellular level. To investigate the potential consequences of somatic heterozygosity for a *BRCA1* mutation demonstrably associated with breast carcinogenesis on background somatic mutational burden, we applied the two standard assays of in vivo human somatic mutation to blood samples from a manifesting carrier of the Q1200X mutation in *BRCA1* whose tumor was uniquely ascertained through an MRI screening study. The patient had an allele-loss mutation frequency of 19.4 x 10^{-6} at the autosomal *GPA* locus in erythrocytes and 17.1 x 10^{-6} at the X-linked *HPRT* locus in lymphocytes. Both of these mutation frequencies are significantly higher than expected from age-matched disease-free controls ($P < 0.05$). Mutation at the *HPRT* locus was similarly elevated in lymphoblastoid cell lines established from three other *BRCA1* mutation carriers with breast cancer. Our patient's *GPA* mutation frequency is below the level established for diagnosis of homozygous Fanconi anemia patients, but consistent with data from obligate heterozygotes. The increased *HPRT* mutation frequency is more reminiscent of data from patients with xeroderma pigmentosum, a disease characterized by UV sensitivity and deficiency in the nucleotide excision pathway of DNA repair. Therefore, this *BRCA1*-associated breast cancer patient manifests a unique phenotype of increased background mutagenesis that likely contributed to the development of her disease independent of loss of heterozygosity at the susceptibility locus. (Pathology Oncology Research Vol 13, No 4, 276–283)

Key words: somatic mutation, hypoxanthine-guanine phosphoribosyl transferase, glycophorin A, *BRCA1* gene, inherited breast cancer syndrome, Fanconi anemia, xeroderma pigmentosum

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Abbreviations/acronyms: AT, ataxia telangiectasia; FA, Fanconi anemia; GPA, glycophorin A; HNPCC, hereditary non-polyposis colorectal cancer; HPRT, hypoxanthine-guanine phosphoribosyltransferase; M_f, mutation frequency; MRI, magnetic resonance imaging; NER, nucleotide excision repair; XP, xeroderma pigmentosum

Introduction

One of the greatest advances in our understanding of cancer etiology has been the identification of subsets of patients exhibiting hereditary susceptibility to the disease. Cancer susceptibility occurs in two distinct forms. The first involves very rare, usually recessive disorders with very high cancer incidence that often have a spectrum of developmental symptoms detectable at birth or in early childhood. Examples include such syndromes as ataxia telangiectasia (AT), Fanconi anemia (FA) and xeroderma pigmentosum (XP), diseases that have been known for decades. Cloning and characterization of the underlying

genetic defects in these diseases has shown that they are associated with deficiencies in the various processes of DNA repair. The second type of cancer susceptibility is more subtle, usually associated with cancer incidence in early adulthood, with few or no other phenotypic manifestations. These diseases are considered to be genetically dominant and, as they occur in patients with no other symptoms, their tumors are often considered to be sporadic in the absence of a considerable family history or molecular analysis. Examples of these disorders include Li-Fraumeni syndrome and Lynch syndrome II or hereditary nonpolyposis colorectal cancer (HNPCC). The underlying defect in these diseases has been identified as heterozygosity for inactivation of recessive oncogenes known as tumor suppressors. Thus, although the tumor-promoting phenotype is recessive at the cellular level, the high incidence of somatic segregation of heterozygous alleles causes it to be effectively dominant at the organismal level. For many years it has been speculated that there might be some connection between these two types of cancer susceptibility.

The breast cancer susceptibility syndromes associated with mutational inactivation of the *BRCA1* and *BRCA2* genes are considered to be of the second type of cancer-prone diseases, almost by definition establishing these genes as tumor suppressors. They have also been implicated in DNA repair processes, however, suggesting that they may, in fact, be "mutator" genes like those responsible for the recessive cancer syndromes.[1] This situation became clearer when it was recently discovered that homozygosity for mutations in the *BRCA2* gene was responsible for FA patients of complementation group D, identifying it also as the *FANCD1* gene.[2] The tumor suppressor genes that are the genetic basis of HNPCC are known to be involved in post-replicative repair of base mismatches in DNA[3] and the p53 gene, the basis of Li-Fraumeni syndrome, is involved in repressing gene amplification,[4] so both of these syndromes could be considered as "mutator" gene syndromes as well.

The possibility that *BRCA1* heterozygotes manifest a cellular phenotype promoting carcinogenesis has been investigated with conflicting results; most studies showing no hypermutability in these cells or individuals,[5,6] while others have reported high spontaneous frequencies of mutation[7] and/or hyperinducibility.[8]

There are two widely applied methods of measuring somatic mutation in humans, both blood cell-based.[9] The clonogenic *HPRT* assay involves chemical selection for inactivation of the X-linked housekeeping gene hypoxanthine-guanine phosphoribosyltransferase in cultured lymphocytes. The flow cytometric glycophorin A (*GPA*) assay is based on detection and quantitation of somatic "allele loss" mutations at the glycophorin A locus on chromosome 4. The *GPA* assay is potentially sensitive to a broad spectrum of mutational mechanismus, including point mutation, small insertions and/or deletions, chromosomal aneuploidy, epigenetic gene inactivation, homologous or nonhomologous recombination.[10] The *HPRT* assay is sensitive to point mutations, small insertions and deletions, but large deletions and chromosome loss are inviable, and recombination appears to be suppressed, probably due to X-inactivation.

Previous studies have shown an association between human in vivo somatic mutation level and elevated risk of cancer,[11-14] particularly in the so-called "DNA repair deficiency" diseases AT, FA and Bloom syndrome, which show 10-, 50- and 100-fold increases in *GPA* mutation frequencies, respectively.[15] Epidemiological evidence also suggests that the otherwise asymptomatic heterozygotes for the radiation-sensitivity syndrome AT are predisposed to breast cancer.[16,17] We recently reported the detection of a mammographically undetectable breast tumor in an MRI pilot screening program of young, high-risk women.[18] In the present study, mutation frequencies (M_f) at the *GPA* and *HPRT* reporter loci are measured in this breast cancer patient, who is now known to be a carrier for an inactivating mutation of the *BRCA1* gene.

Materials and Methods

The patient was a 36-year-old woman with strong family history of breast cancer (two affected first degree relatives) recruited into a clinical trial of MRI screening for young woman at high risk for breast cancer with dense breast tissue.[18] She was subsequently found to carry a Q1200X premature termination mutation in the *BRCA1* locus.[19] Despite recent negative mammography, gadolinium enhancement images revealed a small, 1 cm lesion in the upper-outer quadrant of the left breast, identified pathologically as an infiltrating ductal carcinoma. The patient underwent a modified radical mastectomy of the left breast and chose to also undergo a contralateral prophylactic total mastectomy. Blood was obtained for analysis with consent under Magee-Womens Hospital/University of Pittsburgh IRB # MWH-94-108.

HPRT and GPA somatic mutation analyses

The *HPRT* and *GPA* somatic mutation assays were performed on blood samples obtained from the patient and concurrent disease-free controls. The clonogenic *HPRT* assay was performed by the method of O'Neill et al.[20] T lymphocytes were stimulated to proliferate in culture and enzyme-deficient mutant clones selected in the presence of the toxic nucleoside analog 6-thioguanine. Results for the concurrently analyzed normal control, an M_f of 5.0×10^{-6}, were consistent with our previous experience with this individual ($P = 0.45$, z test), who has been analyzed 6 other

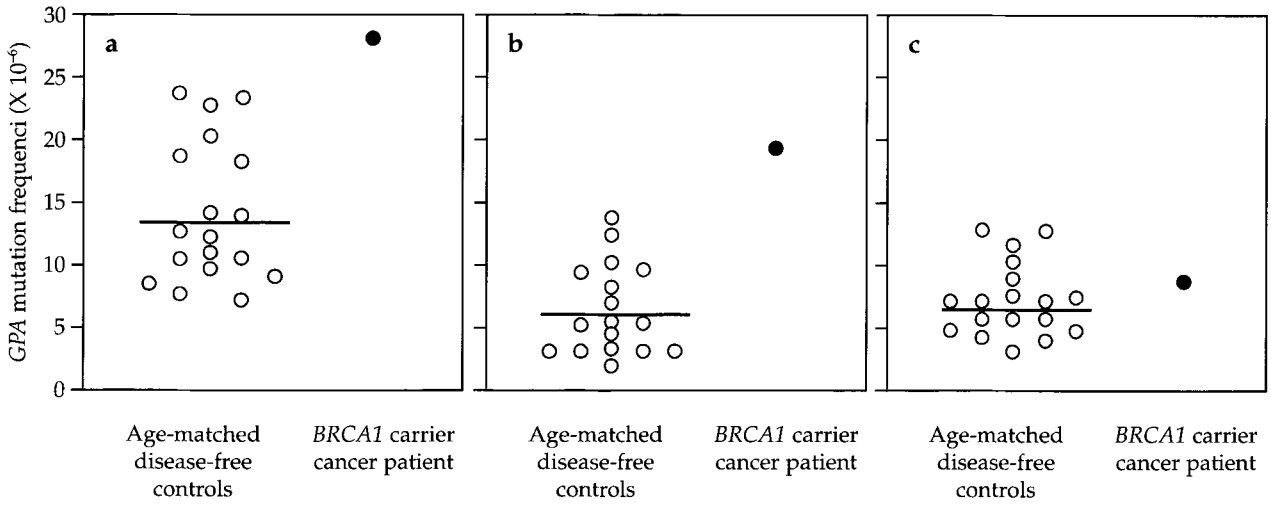

Figure 1. *GPA M_f of the hereditary breast cancer patient and 18 age-matched controls (35-39 years).* ***(a)*** *Total GPA: bar indicates average M_f of control population, 14.0 ± 5.6 x 10^{-6}. The patient's result is significantly higher than the M_f of this limited control population (P = 0.005).* ***(b)*** *Allele loss: bar indicates average M_f of control population, 6.6 ± 3.4 x 10^{-6}. The patient's M_f is significantly higher than that of the controls (P = 0.0001).* ***(c)*** *Loss and duplication: bar indicates average M_f of the control population, 7.4 ± 2.8 x 10^{-6}. The patient's result is not significantly different from the M_f of these controls (P = 0.30).*

times in our laboratory, yielding an average M_f of 8.6 ± 3.3 x 10^{-6} (mean ± standard deviation).

Briefly, the *GPA* assay involves labeling of the two polymorphic forms of the erythrocyte cell-surface protein glycophorin A with monoclonal antibodies followed by flow cytometric analysis to quantify cells with variant phenotypes associated with bone marrow somatic mutation.[21] The patient had the most informative genotype for this analysis, heterozygosity for the MN blood group. Results for the concurrently analyzed normal control (total *GPA* M_f, 12.6 x 10^{-6}, allele loss 6.0 x 10^{-6}, loss and duplication 6.6 x 10^{-6}) were consistent with our previous experience with this individual (P = 0.17, 0.28 and 0.17 for total *GPA* M_f, allele loss frequency and loss and duplication frequency, respectively, z tests), who has been analyzed 11 other times in our laboratory, yielding a total *GPA* M_f of 9.5 ± 1.3 x 10^{-6}, an allele loss frequency of 5.1 ± 0.8 x 10^{-6} and a loss and duplication frequency of 4.4 ± 0.8 x 10^{-6}.

Controls

Normal *GPA* M_f for comparison were derived from our control database, which is under continuous revision and supplementation, and contains historical as well as contemporaneous data. Several subsets of these data have been published.[22-24] Normal *HPRT* M_f for comparison are derived from a number of published reports[25-31] as well as our own contemporaneous data. *GPA* M_f from homozygous and heterozygous FA patients were taken from the report of Sala-Trepat et al.[32] and our own ongoing analyses[33,34] of clinical samples and samples provided from the International Fanconi Anemia Registry (IFAR). *HPRT* M_f from homozygous and heterozygous FA patients were derived from Vijayalaxmi et al.[35] and Sala-Trepat et al.[32] *GPA* allele loss M_f from XP homozygotes were reported in Langlois et al.[15] *HPRT* M_f in XP homozygotes and heterozygotes were compiled from a number of reports.[12,36-42]

Lymphoblastoid cell lines

Three lymphoblastoid cell lines from breast cancer patients confirmed as heterozygous for inactivating mutations in the *BRCA1* gene were obtained from the Coriell Cell Repositories (Camden, NJ): GM13708, GM13709 and GM13712, as well as 9 control cell lines: AG09393, AG09980, AG10111, GM00946, GM01814, GM03797, GM05380, GM14448 and GM14820.

Statistical analysis

Analysis of individual results in the context of control populations was done with the z test at α = 0.05 on ln-transformed data. Comparisons between populations were performed with the t test assuming unequal variances on similarly transformed data or the nonparametric Mann Whitney U test at the same level of significance.

Results

Somatic mutation at the autosomal GPA *locus*

The patient's total *GPA* M_f was found to be 28.2 x 10^{-6}. M_f at this locus are known to be significantly age-dependent,[22,43] so the patient's result is compared with normal women ± 2 years from her age in *Fig. 1*, and against the

Table 1. **GPA mutation frequencies for normal and DNA repair-deficient patients and populations**

		GPA M_f *(x* 10^{-6}*)*		*Patient*	
Population	*N*	*Mean ± SD*	*Range*	*Percentile*	P^1
a) all mutant classes					
Patient		28.2			
Normal controls	644	16.6 ± 16.3[2]	0.19–79.3	0.91	0.22
FA patients	63	455.8 ± 751.6[3]	49.4–4257.4	0.02	0.022
FA carriers	36	31.9 ± 36.7[4]	6.2–90.2	0.66	0.42
b) allele loss mutants only					
Patient		19.4			
Normal controls	644	6.7 ± 4.3[5]	0.01–34.5	0.97	0.001
FA patients	63	379.9 ± 737.9[6]	24.6–4084.0	0.02	0.047
FA carriers	36	10.4 ± 8.0[7]	1.0–37.4	0.90	0.11
XP patients	7	4.6 ± 4.3	1.0–2.2	1.00	0.028
c) allele loss and duplication mutants only					
Patient		8.8			
Normal controls	644	9.2 ± 11.8[8]	0.01–152.3	0.67	0.47
FA patients	63	74.0 ± 56.3[9]	6.2–270.4	0.03	0.016
FA carriers	36	20.5 ± 31.7[10]	4.0–152.8	0.43	0.31

[1] *z*-test for patient data [2] excluding outlier with total *GPA* M_f of 6.7 x 10^{-3} [3] excluding outliers with total *GPA* M_f of 7.4, 8.0 and 9.9 x 10^{-3} [4] excluding outlier with total *GPA* M_f of 2.7 x 10^{-4} [5] excluding 6 outliers with *GPA* allele loss M_f of 4.5, 4.8, 7.1, 7.7, 7.8, and 16.2 x 10^{-5} [6] excluding 3 outliers with *GPA* allele loss M_f of 7.0, 7.9 and 9.8 x 10^{-3} [7] excluding outlier with *GPA* allele loss M_f of 4.4 x 10^{-5} [8] excluding outlier with *GPA* loss and duplication M_f of 8.7 x 10^{-4} [9] excluding 2 outliers with *GPA* loss and duplication M_f of 3.2 and 3.5 x 10^{-4} [10] excluding outlier with *GPA* loss and duplication M_f of 2.6 x 10^{-4}

M_f of our entire normals database, age-adjusted, in *Table 1*. Although she appears to have a somewhat elevated total *GPA* M_f (*Fig. 1*), in the 91st centile for the entire control database, it is not significantly higher than that expected for her age (*Table 1*). The *GPA* assay also allows for the straightforward characterization of mutations into two broad classes; those arising by simple allele loss and those arising by allele loss accompanied by duplication of the remaining allele.[9,10,21] The patient's allele loss M_f was 19.4 x 10^{-6}, and her loss and duplication M_f was 8.8 x 10^{-6} (note that the two add up to her total *GPA* M_f). It can now be seen that the elevation in the patient's M_f is confined to the allele loss class of variation, where it is unambiguously significant (*Fig. 1* and *Table* 2).

Somatic mutation at the X-linked HPRT *locus*

The patient's *HPRT* M_f was found to be 17.1 x 10^{-6}. Once again, in vivo M_f at the *HPRT* locus are known to be age-dependent,[25,44,45] so the patient's result is compared to normal women ± 2 years from her age in *Fig. 2*, and against the M_f of our entire normals database, age-adjusted, in *Table 2*. Compared to the subset of age-matched controls, the patient's M_f does not appear to be unusually high (*Fig. 2*), but in comparison with the entire database it does just reach significance (*Table* 2). To confirm this result, we obtained lymphoblastoid cell lines from 9 disease-free controls and 3 additional breast cancer patients with known inactivating mutations in the *BRCA1* gene (all nonsense mutations resulting in premature truncation of the protein product). The *HPRT* M_f for the normal cell lines was 8.8 ± 6.8 x 10^{-6}, not significantly different from our in vivo controls ($P = 0.69$). The mean *HPRT* M_f for the 3 heterozygous *BRCA1* patient cell lines (*Fig. 2*) was 16.7 ± 1.2 x 10^{-6}, not significantly different from the in vivo result from our patient ($P = 0.63$), but signification higher than that of the in vitro controls ($P = 0.009$). This effect is accentuated by combining the in vivo and in vitro data ($P < 0.0001$).

Discussion

In vivo somatic M_f in a breast cancer patient heterozygous for an inactivating mutation in the *BRCA1* gene was significantly elevated over disease-free controls; 1.7-fold in the case of *GPA* M_f and 2.3-fold in the case of in vivo *HPRT* M_f. The latter result was confirmed in a study of lymphoblastoid cell lines derived from breast cancer patients, which exhibited a 1.9-fold increase in *HPRT* M_f over similar controls. These increases are similar to those observed in analyses of M_f in blood samples from sporadic

cancer patients: 1.7-fold increases in both *HPRT*[12] and *GPA* M_f.[14] In breast cancer patients these increases in M_f are 1.3-fold for *HPRT*[46] and 1.5-fold for *GPA*.[47] In our hereditary breast cancer patient, this increase becomes 2.9-fold if only the "allele loss" class of *GPA* mutation is considered, a pattern that is consistent with induced mutation by most genotoxic exposures, including ionizing radiation,[9] and the elevated spontaneous M_f observed in patients with AT.[24] This pattern suggests that haploinsufficiency for *BRCA1* does not affect the incidence of mitotic recombination (including gene conversion) or chromosome missegregation, as they result in allele loss and duplication. Possible mechanisms associated with the observed increase in simple allele loss still range from point mutations and small, intragenic insertions and deletions to large-scale, cytogenetically detectable chromosome deletion and even whole chromosome loss,[10,21] however, since we observed a comparable increase in mutation frequency at the X-linked *HPRT* locus, and major deletions and chromosome loss are inviable at this locus,[48] it would appear that *BRCA1* heterozygosity is associated with gene-specific rather than regional mutational susceptibility.

***Table 2.* HPRT mutation frequencies for normal and DNA repair-deficient patients and populations**

		HPRT M_f ($\times 10^{-6}$)		*Patient*	
Population	*N*	*Mean ± SD*	*Range*	*Percentile*	*P*[1]
Patient		17.1			
Normal controls	482	7.5 ± 4.9[2]	0.32–6.4	0.95	0.049
FA patients	28	5.8 ± 4.6[3]	0.20–5.4	0.97	0.083
FA carriers	27	12.0 ± 5.0[4]	5.0–5.0	0.90	0.13
XP patients	16	15.0 ± 10.7	1.8–6.0	0.65	0.29
XP carriers	7	11.9 ± 8.7	3.4–0.4	0.88	0.19

[1] *z*-test for patient data [2] excluding 10 outliers with *HPRT* M_f of > 3.1×10^{-5} [3] excluding outlier with *HPRT* M_f of 2.6×10^{-5} [4] excluding outlier with *HPRT* M_f of 6.8×10^{-5}

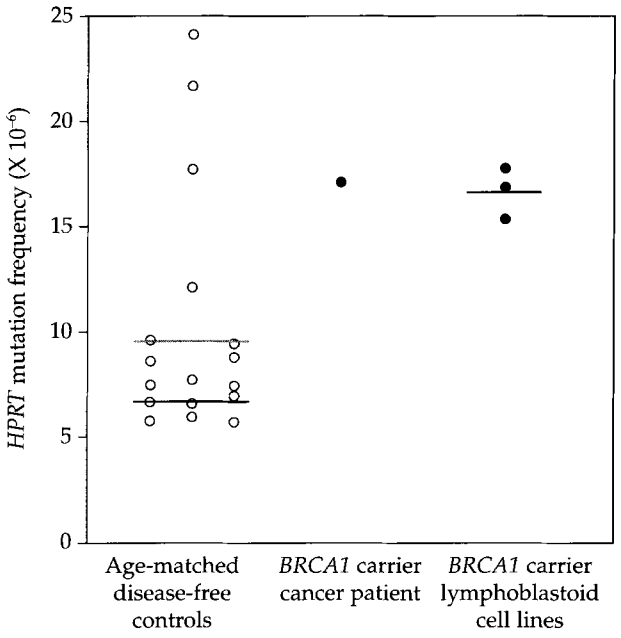

Figure 2. *HPRT M_f of the hereditary breast cancer patient, 18 age-matched controls (35-39 years), 3 lymphoblastoid cell lines derived from hereditary breast cancer patients and 9 lymphoblastoid cell lines derived from disease-free controls. The solid bars represent the average M_f for the control population ($9.7 \pm 5.8 \times 10^{-6}$), heterozygous BRCA1$^{+/-}$ cell lines ($16.7 \pm 1.2 \times 10^{-6}$), and control cell lines ($8.8 \pm 6.8 \times 10^{-6}$), respectively. The patient's result itself is not significantly higher than that of this restricted set of controls ($P = 0.092$), but the average M_f of the 3 patient-derived lymphoblastoid cell lines is, both alone ($P = 0.048$) and with the addition of the patient's lymphocyte-derived data ($P = 0.022$). The lighter line represents the average M_f of the entire control database age-adjusted to that of the patient (Table 2).*

In patients with FA, we have found a consistent increase in allele loss M_f, but a variable increase in the frequency of loss and duplication.[33,34] Since the *BRCA1* gene has been functionally linked to the FA repair system,[1] deficiency for this gene might be expected to produce a similar phenotype. As can be seen from Table 1, however, the *GPA* M_f of our *BRCA1* heterozygote was significantly lower than those seen in FA patients, both in total and in each mutational subclass. A more valid comparison, however, is to compare the patient's results to those of FA heterozygotes, who do seem to have a subtle mutational susceptibility phenotype.[32-34] While the overall increase in *GPA* M_f in our patient is certainly consistent with that observed in FA heterozygotes, the pattern does not match, with the patient's increase occurring in the allele loss class and the FA heterozygotes primarily increased in loss and duplication M_f.

Somewhat surprisingly, FA patients do not exhibit an increased M_f at the *HPRT* locus,[32] although they have a significant shift in their *HPRT* mutational spectrum towards gene deletions.[49,50] These two apparently contradictory findings are rationalized by invoking the well-established limited viability of these cells, especially in the presence of genotoxic agents.[51,52] Indeed, in the present analysis FA patients actually exhibit a significantly lower *HPRT* M_f than controls ($P = 0.037$) (Table 2). FA heterozygotes, however, exhibit an increased *HPRT* M_f, which, although not significant in either of the original reports,[32,35] is highly significant in our pooled analysis ($P <$

0.001). This increase in *HPRT* M_f is consistent with those observed in our *BRCA1* carriers, both in vivo (*Table 2*) and in vitro ($P = 0.10$).

Another possible explanation for the increased mutation frequency we have observed in *BRCA1* heterozygotes is an effect on the nucleotide excision DNA repair pathway (NER). This pathway repairs DNA damage that causes a distortion in the DNA helix, notably intrastrand linkages caused by UV irradiation and bulky adducts such as those caused by exposure to polycyclic aromatic hydrocarbons, and deficiency in this DNA repair system is associated with the cancer-predisposing disease XP.[53] Overexpression of *BRCA1* has been shown to enhance NER capacity,[54] probably through transcriptional regulation of NER genes.[55] Several studies have suggested that breast cancer patients have lower NER capacity than the normal population,[56-58] and we have shown that early stage breast tumors are consistently deficient in this type of DNA repair.[47]

The phenotype of XP patients seems to be the opposite of that of FA patients: they exhibit significantly increased *HPRT* M_f,[12,36-42] but there is no evidence of an effect on *GPA* M_f[15] (*Tables 1 and 2*). In our pooled analysis, the increase in *HPRT* M_f is highly significant ($P = 0.008$), and is consistent with that of our *BRCA1* carrier patient (*Table 2*) and data from the heterozygous *BRCA1* lymphoblastoid cell lines ($P = 0.23$). XP heterozygotes also exhibit a slightly increased *HPRT* M_f,[37-39] although it does not reach statistical significance ($P = 0.16$). Since XP patients have been identified with as much as 50% residual NER activity,[53] it is reasonable that heterozygotes should express a detectable phenotype, but, since the *HPRT* M_f increase in XP homozygotes is only on the order of 2-fold it would require analysis of a much larger population of heterozygotes to achieve statistical significance. Again, however, the results from our patient (*Table 2*), and the *BRCA1* carrier cell lines ($P = 0.22$) are not significantly different from those of the population of XP carriers.

BRCA1 carriers therefore manifest a unique phenotype with respect to mutational susceptibility that may be related to their tumor incidence. A more significant deficiency in *BRCA1* activity arises during tumorigenesis when the locus undergoes loss of heterozygosity, but this is likely to play a greater role in tumor progression.

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References

1. *D'Andrea AD, Grompe M*: The Fanconi anaemia/BRCA pathway. Nat Rev Cancer 3: 23-34, 2003
2. *Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea AD*: Biallelic inactivation of BRCA2 in Fanconi anemia. Science 297: 606-609, 2002
3. *Papadopoulos N, Lindblom A*: Molecular basis of HNPCC: mutations of MMR genes. Hum Mutat 10: 89-99, 1997
4. *Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD*: Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70: 923-935, 1992
5. *Nieuwenhuis B, Van Assen-Bolt AJ, Van Waarde-Verhagen MA, Sijmons RH, Van der Hout AH, Bauch T, Streffer C, Kampinga HH*: BRCA1 and BRCA2 heterozygosity and repair of X-ray-induced DNA damage. Int J Radiat Biol 78: 285-295, 2002
6. *Trenz K, Rothfuss A, Schutz P, Speit G*: Mutagen sensitivity of peripheral blood from women carrying a BRCA1 or BRCA2 mutation. Mutat Res 500: 89-96, 2002
7. *Mamon H, Dahlberg W, Little JB*: Hemizygous fibroblast cell strains established from patients with BRCA1 or BRCA2 mutations demonstrate an increased rate of spontaneous mutations and increased radiosensitivity. Int J Radiat Oncol Biol Phys 57(Suppl 2): S346-S347, 2003
8. *Rothfuss A, Schutz P, Bochum S, Volm T, Eberhardt E, Kreienberg R, Vogel W, Speit G*: Induced micronucleus frequencies in peripheral lymphocytes as a screening test for carriers of a BRCA1 mutation in breast cancer families. Cancer Res 60: 390-394, 2000
9. *Grant SG, Jensen RH*: Use of hematopoietic cells and markers for the detection and quantitation of human *in vivo* somatic mutation. In: Immunobiology of Transfusion Medicine. (Ed: Garratty G), Marcel Dekker, New York, 1993, pp. 299-323
10. *Grant SG, Bigbee WL, Langlois RG, Jensen RH*: Methods for the detection of mutational and segregational events: relevance to the monitoring of survivors of childhood cancer. In: Late Effects of Treatment for Childhood Cancer. (Eds: Green DM, and D'Angio GJ), Wiley-Liss, New York, 1992, pp. 133-150
11. *Tompa A, Sapi E*: Detection of 6-thioguanine resistance in human peripheral blood lymphocytes (PBL) of industrial workers and lung cancer patients. Mutat Res 210: 345-351, 1989
12. *Cole J, Skopek TR*: Somatic mutant frequency, mutation rates and mutational spectra in the human population in vivo. Mutat Res 304: 33-105, 1994
13. *Okada S, Ishii H, Nose H, Okusaka T, Kyogoku A, Yoshimori M, Wakabayashi K*: Evidence for increased somatic cell mutations in patients with hepatocellular carcinoma. Carcinogenesis 18: 445-449, 1997
14. *Grant SG*: Molecular epidemiology of human cancer: biomarkers of genotoxic exposure and susceptibility. J Environ Pathol Toxicol Oncol 20: 245-261, 2001
15. *Langlois RG, Bigbee WL, Jensen RH*: The glycophorin A assay for somatic cell mutations in humans. Prog Clin Biol Res 340C: 47-56, 1990
16. *Swift M, Morrell D, Massey RB, Chase CL*: Incidence of cancer in 161 families affected by ataxia-telangiecasia. N Engl J Med 325: 1831-1836, 1991
17. *Kerangueven F, Eisinger F, Noguchi T, Allione F, Wargniez V, Eng C, Padberg G, Theillet C, Jacquemier J, Longy M, Sobol H, Birnbaum D*: Loss of heterozygosity in human breast carcinomas in the ataxia telangiectasia, Cowden disease and BRCA1 regions. Oncogene 14: 339-347, 1997

18. *Rubinstein WS, Latimer JJ, Sumkin JH, Huerbin M, Grant SG, Vogel VG*: Prospective screening study of 0.5 Tesla dedicated magnetic resonance imaging for the detection of breast cancer in young, high risk women. BMC Women's Health 6: 10, 2006
19. *Latimer JJ, Rubinstein WS, Johnson JM, Kanbour-Shakir A, Vogel VG, Grant SG*: Haploinsufficiency for *BRCA1* is associated with normal levels of DNA nucleotide excision repair in breast tissue and blood lymphocytes. BMC Med Genet 6: 26, 2005
20. *O'Neill JP, McGinniss MJ, Berman JK, Sullivan LM, Nicklas JA, Albertini RJ*: Refinement of a T-lymphocyte cloning assay to quantify the *in vivo* thioguanine-resistant mutant frequency in humans. Mutagenesis 2: 87-94, 1987
21. *Grant SG*: The *GPA* in vivo somatic mutation assay. Meth Mol Biol 291: 179-195, 2005
22. *Jensen RH, Bigbee WL, Langlois RG*: In vivo somatic mutations in the glycophorin A locus of human erythroid cells. Banbury Rep 28: 149-159, 1987
23. *Manchester DK, Nicklas JA, O'Neill JP, Lippert MJ, Grant SG, Langlois RG, Moore DH 3rd, Jensen RH, Albertini RJ, Bigbee WL*: Sensitivity of somatic mutations in human umbilical cord blood to maternal environments. Environ Mol Mutagen 26: 203-212, 1995
24. *Grant SG, Reeger W, Wenger SL*: Diagnosis of ataxia telangiectasia with the glycophorin A somatic mutation assay. Genet Testing 1: 261-267, 1998
25. *Finette BA, Sullivan LM, O'Neill JP, Nicklas JA, Vacek PM, Albertini RJ*: Determination of *hprt* mutant frequencies in T-lymphocytes from a healthy pediatric population: statistical comparison between newborn, children and adult mutant frequencies, cloning efficiency and age. Mutat Res 308: 223-231, 1994
26. *Tates AD, van Dam FJ, van Mossel H, Schoemaker H, Thijssen JC, Woldring VM, Zwinderman AH, Natarajan AT*: Use of the clonal assay for the measurement of frequencies of HPRT mutants in T-lymphocytes from five control populations. Mutat Res 253: 199-213, 1991
27. *Branda RF, Sullivan LM, O'Neill JP, Falta MT, Nicklas JA, Hirsch B, Vacek PM, Albertini RJ*: Measurement of HPRT mutant frequencies in T-lymphocytes from healthy human populations. Mutat Res 285: 267-279, 1993
28. *Jones IM, Moore DH, Thomas CB, Thompson CL, Strout CL, Burkhart-Schultz K*: Factors affecting *HPRT* mutant frequency in T-lymphocytes of smokers and nonsmokers. Cancer Epidemiol Biomarkers Prev 2: 249-260, 1993
29. *Finette BA, Kendall H, Vacek PM*: Mutational spectral analysis at the HPRT locus in healthy children. Mutat Res 505: 27-41, 2002
30. *Hüttner E, Holzapfel B, Kropf S*: Frequency of HPRT mutant lymphocytes in a human control population as determined by the T-cell cloning procedure. Mutat Res 348: 83-91, 1995
31. *Becker R, Nikolova T, Wolff I, Lovell D, Hüttner E, Foth H*: Frequency of HPRT mutants in humans exposed to vinyl chloride via an environmental accident. Mutat Res 494: 87-96, 2001
32. *Sala-Trepat M, Boyse J, Richard P, Papadopoulo D, Moustacchi E*: Frequencies of *HPRT*⁻ lymphocytes and glycophorin A variants erythrocytes in Fanconi anemia patients, their parents and control donors. Mutat Res 289: 115-126, 1993
33. *Evdokimova VN, McLoughlin RK, Wenger SL, Grant SG*: Use of the glycophorin A bone marrow somatic mutation assay for rapid, unambiguous identification of Fanconi anemia homozygotes regardless of *GPA* genotype. Am J Med Genet 135: 59-65, 2005
34. *Grant SG, Wenger SL, Rubinstein WS, Latimer JJ, Bigbee WL, Auerbach AD*: Elevated levels of somatic mutation in homozygotes and heterozygotes for inactivating mutations in the genes of the FA/BRCA DNA repair pathway [abstract]. Am J Hum Genet 75(Suppl): 94, 2004
35. *Vijayalaxmi, Wunder E, Schroeder TM*: Spontaneous 6-thioguanine-resistant lymphocytes in Fanconi anemia patients and their heterozygous parents. Hum Genet 70: 264-270, 1985
36. *Tates AD, Bernini LF, Natarajan AT, Ploem JS, Verwoerd NP, Cole J, Green MHL, Arlett CF, Norris PN*: Detection of somatic mutants in man: HPRT mutations in lymphocytes and hemoglobin mutations in erythrocytes. Mutat Res 213: 73-82, 1989
37. *Cole J, Green MHL, Stephens G, Waugh AP, Beare D, Steingrimsdottir H, Brideges BA*: HPRT somatic mutation data. Prog Clin Biol Res 340C: 25-35, 1990
38. *Norris PG, Limb GA, Hamblin AS, Lehmann AR, Arlett CF, Cole J, Waugh AP, Hawk JL*: Immune function, mutant frequency, and cancer risk in the DNA repair defective genodermatoses xeroderma pigmentosum, Cockayne's syndrome, and trichothiodystrophy. J Invest Dermatol 94: 94-100, 1990
39. *Vermeulen W, Scott RJ, Rodgers S, Muller HJ, Cole J, Arlett CF, Kleijer WJ, Bootsma D, Hoeijmakers JH, Weeda G*: Clinical heterogeneity within xeroderma pigmentosum associated with mutations in the DNA repair and transcription gene *ERCC3*. Am J Hum Genet 54: 191-200, 1994
40. *Anstey AV, Arlett CF, Cole J, Norris PG, Hamblin AS, Limb GA, Lehmann AR, Wilkinson JD, Turner M*: Long-term survival and preservation of natural killer cell activity in a xeroderma pigmentosum patient with spontaneous regression and multiple deposits of malignant melanoma. Br J Dermatol 125: 272-278, 1991
41. *Cole J, Arlett CF, Norris PG, Stephens G, Waugh AP, Beare DM, Green MHL*: Elevated *hprt* mutant frequency in circulating T-lymphocytes of xeroderma pigmentosum patients. Mutat Res 273: 171-178, 1992
42. *Lin YW, Kubota M, Hirota H, Furusho K, Tomiwa K, Ochi J, Kasahara Y, Sasaki H, Ohta S*: Somatic cell mutation frequency at the HPRT, T-cell antigen receptor and glycophorin A loci in Cockayne syndrome. Mutat Res 337: 49-55, 1995
43. *Akiyama M, Kyoizumi S, Hirai Y, Kusunoki Y, Iwamoto KS, Nakamura N*: Mutation frequency in human blood cells increases with age. Mutat Res 338: 141-149, 1995
44. *Morley AA, Cox S, Holliday R*: Human lymphocytes resistant to 6-thioguanine increase with age. Mech Ageing Dev 19: 21-26, 1982
45. *Vrieling H, Tates AD, Natarajan AT, van Zeeland AA*: Age-related accumulation of mutations in human T-lymphocytes. Ann N Y Acad Sci 663: 36-42, 1992
46. *Branda RF, O'Neill JP, Jacobson-Kram D, Albertini RJ*: Factors influencing mutation at the *hprt* locus in T-lymphocytes: studies in normal women and women with benign and malignant breast masses. Environ Mol Mutagen 19: 274-281, 1992
47. *Latimer JJ, Kisin E, Zayas-Rivera B, Kanbour-Shakir A, Kelley J, Johnson R, Grant SG*: Increased somatic mutation and reduced DNA repair in breast cancer patients and their tumors [abstract]. Proc Am Assoc Cancer Res 40: 440, 1999
48. *Nicklas JA, Lippert MJ, Hunter TC, O'Neill JP, Albertini RJ*: Analysis of human *HPRT* deletion mutations with X-linked probes and pulsed field gel electrophoresis. Environ Mol Mutagen 18: 270-273, 1991
49. *Papadopoulo D, Guillouf C, Mohrenweiser H, Moustacchi E*: Hypomutability in Fanconi anemia cells is associated with increased deletion frequency at the HPRT locus. Proc Natl Acad Sci USA 87: 8383-8387, 1990

50. *Laquerbe A, Sala-Trepat M, Vives C, Escarceller M, Papadopoulo D*: Molecular spectra of HPRT deletion mutations in circulating T-lymphocytes in Fanconi anemia patients. Mutat Res 431: 341-350, 1999
51. *Elmore E, Swift M*: Growth of cultured cells from patients with Fanconi anemia. J Cell Physiol 87: 229-233, 1975
52. *Weksberg R, Buchwald M, Sargent P, Thompson MW, Siminovitch L*: Specific cellular defects in patients with Fanconi anemia. J Cell Physiol 101: 311-323, 1979
53. *Thompson LH*: Nucleotide excision repair: its relation to human disease. In: DNA Damage and Repair, Volume 2: DNA Repair in Higher Eukaryotes. (Eds: Nickoloff JA and Hoekstra MF), Humana, Totowa, NJ, 1998, pp. 335-393
54. *Hartman A-R, Ford JM*: BRCA1 induces DNA damage recognition factors and enhances nucleotide excision repair. Nat Genet 32: 180-184, 2002
55. *Takimoto R, MacLachlan TK, Dicker DT, Niitsu Y, Mori T, el-Deiry WS*: BRCA1 transcriptionally regulates damaged DNA binding protein (DDB2) in the DNA repair response following UV-irradiation. Cancer Biol Ther 1: 177-186, 2002 (comment 1: 187-188, 2002)
56. *Kovacs E, Stucki D, Weber W, Muller H*: Impaired DNA-repair synthesis in lymphocytes of breast cancer patients. Eur J Cancer Clin Oncol 22: 863-869, 1986
57. *Xiong P, Bondy ML, Li D, Shen H, Wang LE, Singletary SE, Spitz MR, Wei Q*: Sensitivity to benzo[a]pyrene diol-epoxide associated with risk of breast cancer in young women and modulation by glutathione S-transferase polymorphisms: a case-control study. Cancer Res 61: 8465-8469, 2001
58. *Kennedy DO, Agrawal M, Shen J, Terry MB, Zhang FF, Senie RT, Motykiewicz G, Santella RM*: DNA repair capacity of lymphoblastoid cell lines from sisters discordant for breast cancer. J Natl Cancer Inst 97: 127-132, 2005