Polymorphism in glutathione *S*-transferase P1 is associated with susceptibility to chemotherapy-induced leukemia

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Glutathione *S*-transferases (GSTs) detoxify potentially mutagenic and toxic DNA-reactive electrophiles, including metabolites of several chemotherapeutic agents, some of which are suspected human carcinogens. Functional polymorphisms exist in at least three genes that encode GSTs, including *GSTM1, GSTT1,* and *GSTP1.* We hypothesize, therefore, that polymorphisms in genes that encode GSTs alter susceptibility to chemotherapy-induced carcinogenesis, specifically to therapy-related acute myeloid leukemia (t-AML), a devastating complication of long-term cancer survival. Elucidation of genetic determinants may help to identify individuals at increased risk of developing t-AML. To this end, we have examined 89 cases of t-AML, 420 cases of *de novo* AML, and 1,022 controls for polymorphisms in *GSTM1, GSTT1,* and *GSTP1*. Gene deletion of *GSTM1* or *GSTT1* was not specifically associated with susceptibility to t-AML. Individuals with at least one *GSTP1* codon 105 Val allele were significantly over-represented in t-AML cases compared with *de novo* AML cases [odds ratio (OR), 1.81; 95% confidence interval (CI), 1.11–2.94]. Moreover, relative to *de novo* AML, the *GSTP1* codon 105 Val allele occurred more often among t-AML patients with prior exposure to chemotherapy (OR, 2.66; 95% CI, 1.39–5.09), particularly among those with prior exposure to known GSTP1 substrates (OR, 4.34; 95% CI, 1.43–13.20), and not among those t-AML patients with prior exposure to radiotherapy alone (OR,1.01; 95% CI, 0.50–2.07). These data suggest that inheritance of at least one Val allele at *GSTP1* codon 105 confers a significantly increased risk of developing t-AML after cytotoxic chemotherapy, but not after radiotherapy.

Extensive use of combination chemotherapy and radiation therapy has resulted in increased long-term survival of cancer patients. A life-threatening complication of improved long-term cancer survival is an increased risk of developing a second therapy-related cancer, of which acute myeloid leukemia (AML) is the most common (1–6). The cumulative risk of therapy-related AML (t-AML) at 10 years after treatment for breast cancer, non-Hodgkin's lymphoma, ovarian cancer, or Hodgkin's disease has been estimated at 1.5%, 7.9%, 8.5%, and 3.8%, respectively (7–10).

The cytogenetic and clinical presentation of t-AML differs according to the nature of the primary therapy, suggesting the existence of multiple genetic mechanisms by which t-AML may develop (11). Recent efforts have concentrated on elucidating genetic factors that modulate susceptibility to t-AML. Indeed, germ-line mutations in the tumor suppressor gene *p53* have been associated with increased susceptibility to t-AML (12, 13), as has polymorphic variation in the NAD(P)H:quinone oxidoreductase gene (14, 15) and the cytochrome P450 3A4 gene (16).

Polymorphisms of functional significance have also been reported in genes that encode phase II metabolizing enzymes, including glutathione *S*-transferases (GSTs). GSTs detoxify potentially mutagenic and cytotoxic DNA-reactive metabolites by conjugation to glutathione. There are four cytosolic families of GSTs, including GST α, GST μ, GST θ, and GST π (17). Gene clusters of GST μ *(GSTM1, M2, M3, M4,* and *M5*) and GST θ (*GSTT1* and *T2*) are located on chromosomes 1 and 22, respectively (18, 19). Independent gene deletions exist at both *GSTM1* and *GSTT1* loci, resulting in a lack of active protein in $\approx$50% and 20% of Caucasians, respectively (20, 21). GST π or GSTP1, encoded by a single locus (*GSTP1*) on chromosome 11, is also subject to polymorphic variation (22). Codon 105 residue forms part of the GSTP1 active site for binding of hydrophobic electrophiles (23), and the Ile–Val substitution affects substrate-specific catalytic activity and thermal stability of the encoded protein (24–27).

Polymorphisms within genes that encode GSTs have been associated with susceptibility to nonmalignant (28) and malignant human diseases (29, 30), including AML (31). Presumably, altered cancer risk because of polymorphic variation is mediated by differential ability to conjugate and detoxify both endogenously formed and exogenously derived electrophiles and their metabolites. GSTs, particularly GSTP1, also conjugate and protect against the cytotoxic effects of some chemotherapeutic agents. Reactive metabolites of ifosfamide, busulfan, and chlorambucil are substrates for GSTP1-mediated glutathione conjugation *in vitro* (27, 32, 33). Furthermore, transfection of *GSTP1* gene or antisense expression vectors demonstrates a role in cellular resistance to platinum derivatives, etoposide, cyclophosphamide, melphalan, and adriamycin (34–40).

Several chemotherapeutic agents, including cyclophosphamide, melphalan, adriamycin, and chlorambucil, are suspected human leukemogens. Furthermore, $CD34^+$ bone marrow stem cells, the target cell population for leukemic transformation, can be protected against the cytotoxic effects of these suspected leukemogens by *GSTP1*-gene transduction (37–39). These observations led us to question whether GSTs may also protect bone marrow stem cells against chemotherapy-induced mutagenesis and leukemogenesis. Thus, we hypothesize that polymorphic variation in genes encoding GSTs may alter susceptibility to t-AML. To begin to test this hypothesis, we established a rare-case series of individuals with AML whose leukemia developed subsequent to cytotoxic therapy for a previous condition, and we examined this population for the distribution of polymorphisms in *GSTM1, GSTT1,* and *GSTP1*.

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Abbreviations: AML, acute myeloid leukemia; t-AML, therapy-related AML; GST, glutathione *S*-transferase; MRC, Medical Research Council; OR, odds ratio; CI, confidence interval.

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Materials and Methods

Subjects. Polymorphisms of three groups of subjects were compared; there were 89 cases of t-AML, 420 cases of *de novo* AML, and 1,022 unaffected controls. For this study, t-AML is defined as AML following chemotherapy and/or radiotherapy diagnosed at least 2 months after the start of the initial cytotoxic therapy. All samples from the *de novo* AML and control groups, and 24 of those with t-AML, were routinely obtained as part of a large, population-based, case-control study of acute leukemia that has been fully described elsewhere (31, 41–44). Briefly, all subjects were between 16 and 69 years of age and were diagnosed with AML between April 1991 and December 1996 while resident in parts of the north and southwest of England. All diagnoses were pathologically confirmed. Individuals were considered ineligible if, before a diagnosis of acute leukemia, they had been diagnosed with chronic myeloid leukemia or myelodysplastic syndrome within the previous 6 months, or with any malignancy within the previous 2 years. Two controls per patient, individually matched by sex, age, and ethnic origin, were randomly selected from the general practice where the case was registered.

Additional DNA samples were obtained from 65 subjects with t-AML enrolled in the Medical Research Council (MRC) of the United Kingdom's AML trials 10, 11, or 12 (45). To treat these 65 individuals in a similar manner to those enrolled in the case-control study, each person was individually matched by sex and age (± 3 years, or the nearest age for MRC trial patients over 70 years old) to one of the unused pool of unaffected controls recruited in the main case-control study. However, an unmatched statistical analysis was used in all instances (see below).

DNA Extraction. DNA was extracted either from whole frozen blood (case-control study) or from archived bone marrow smears (MRC cases). Genomic DNA was extracted from whole frozen blood, as previously described (31), and from bone marrow smears by using the Qiamp DNA-extraction minikit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations for archived bone marrow.

GST Genotyping. *GSTM1* and *GSTT1* genotyping was performed by using a multiplex PCR assay as previously described (28). Briefly, the presence of 215-bp and 480-bp amplicons corresponds to individuals with at least one intact *GSTM1* and *GSTT1* allele, respectively. The absence of either of these amplicons corresponds to individuals homozygous for the null allele. The presence of a 268-bp amplicon from the ubiquitous β-globin gene acts as an internal control, confirming successful PCR amplification.

GSTP1 codon 105 genotyping was performed by using a PCR-restriction fragment length polymorphism assay as previously described (28). Briefly, digestion of a 177-bp amplicon with *Bsm*A1 that results in either retention of the 177-bp amplicon or in complete digestion to 93-bp and 84-bp fragments corresponds to individuals homozygous for the Ile or Val alleles, respectively. The presence of all three fragments after digestion corresponds to individuals heterozygous at codon 105.

Statistical Analysis. Odds ratios (ORs) and 95% confidence intervals (CIs), adjusted for age and sex, were estimated by using unconditional logistic regression (46). *GSTP1* was analyzed as a trichotamous (Ile/Ile, Ile/Val, Val/Val) or dichotomous (Ile/Ile, Ile/Val + Val/Val) variable. Chemotherapy was given precedence over radiotherapy; individuals who received both were grouped with those who received chemotherapy alone. Confirmed GSTP1 substrates include the following chemotherapeutic agents wherein GSTP1-catalyzed *in vitro* glutathione conjugation has been demonstrated or where sense or antisense gene expression confers resistance or sensitivity, respectively: chlorambucil (27); busulfan (33); ifosfamide (32); adriamycin (35–38, 40); etoposide (36, 38, 40); cyclophosphamide (37, 39); melphalan (36, 38); and cisplatin derivatives (34, 36, 38). All analyses were conducted by using STATA 1999 (Stata, College Station, TX).

Results

Descriptive Results. Table 1 describes the distribution of the t-AML case series, the *de novo* AML cases, and the controls by sex, age, French–American–British (FAB) acute leukemia classification type, cytogenetic analysis, chromosomal abnormalities, and prior therapy as appropriate. The series of 89 t-AML cases comprised 12 individuals (14%) with a documented history of myelodysplastic syndrome. The remaining 77 individuals presented without a preceding dysplastic phase. The type of chromosomal abnormality and prevalence of aberrations observed differed in t-AML from *de novo* AML; abnormalities involving 11q23 or the loss of 5q or 7q were more prevalent among t-AML patients, as was the occurrence of three or more aberrations (Table 1).

Information on the nature of the primary condition was available for 57 of 89 t-AML cases. Cytotoxic therapy was given for primary malignancies in 50 of these 57 cases. Primary malignancies included breast cancer ($n = 15$), Hodgkin's disease ($n = 11$), non-Hodgkin's lymphoma ($n = 4$), carcinoma of the endometrium ($n = 3$), acute lymphocytic leukemia ($n = 3$), malignant histiocytoma ($n = 2$), prostate cancer ($n = 2$), cervical cancer ($n = 2$), osteosarcoma ($n = 1$), bladder cancer ($n = 1$), testicular cancer ($n = 1$), rectal cancer ($n = 1$), chronic lymphocytic leukemia ($n = 1$), basal cell carcinoma ($n = 1$), and lung cancer ($n = 1$). Before diagnosis of t-AML, one individual received cytotoxic therapy for carcinoma of the endometrium 9 years before and non-Hodgkin's lymphoma 5 years before. The remaining 7 t-AML patients were treated with cytotoxic therapy for nonmalignant conditions that included vasculitis ($n = 2$), thrombocytosis ($n = 2$), tuberculosis ($n = 1$), goiter ($n = 1$), and rheumatoid arthritis ($n = 1$).

Among the 38 t-AML patients with previous exposure to radiotherapy only (Table 1), the median latency period from initial radiotherapy to diagnosis of AML was 72 months (based on 23 cases with data available). Fifty-one t-AML patients had previous exposure to chemotherapy and 19 of these had previous exposure to both chemotherapy and radiotherapy; the median latency period from initial chemotherapy to onset of AML was 62.5 months (based on 38 cases with data available). Among the 35 individuals for whom the usage of specific chemotherapeutic agents was documented, the majority ($n = 30$) had prior exposure to combination chemotherapy, for which alkylating agents were the most common components used (Table 1).

GST Genotype and Risk of t-AML. Although the frequencies of *GSTM1* and *GSTT1* null polymorphisms are elevated in the t-AML patient series relative to the population controls, the risks for both polymorphisms are equally raised among *de novo* AML patients (Table 2), suggesting an increased risk of developing acute myeloid leukemia *per se* (31). In contrast, individuals with at least one *GSTP1* Val allele (Ile/Val or Val/Val) at codon 105 are significantly overrepresented among t-AML patients (63%) but not among *de novo* AML patients (51%) compared with population controls (51%), suggesting an increased risk of developing t-AML specifically (OR 1.58, 95% CI 1.01–2.49) (Table 2). Thus, individuals with at least one *GSTP1* codon 105 Val allele are significantly overrepresented in the t-AML patient series compared with *de novo* AML patients (OR 1.81, 95% CI 1.11–2.94) (Table 3).

Table 1. Description of t-AML cases, *de novo* AML cases, and controls

Variable	t-AML cases, *n* (%)	*De novo* AML cases, *n* (%)	Controls, *n* (%)
Total	89 (100)	420 (100)	1,022 (100)
Sex			
Male	36 (40)	227 (54)	538 (53)
Female	53 (60)	193 (46)	484 (47)
Age, years			
Mean (range)	55.3 (17.4–77.8)	47.5 (16.2–70.0)	48.6 (16.2–69.9)
Ethnic group			
United Kingdom Caucasian	69 (78)	420 (100)	1,022 (100)
Not known	20 (22)	—	—
FAB Type*			
M0	2 (2)	21 (5)	—
M1	10 (11)	75 (18)	—
M2	15 (17)	97 (23)	—
M3	11 (12)	53 (13)	—
M4	15 (17)	71 (17)	—
M5	9 (10)	37 (9)	—
M6	5 (6)	18 (4)	—
M7	3 (3)	4 (1)	—
Not known	19 (21)	44 (10)	—
Cytogenetic investigation			
Successful	68 (76)	341 (81)	—
Failed	2 (2)	23 (5)	—
Not done	19 (21)	56 (13)	—
Chromosomal abnormalities†			
No abnormality	22 (32)	149 (44)	—
Any abnormality	46 (68)	192 (56)	—
t(15;17)(q22;q12)	8 (12)	44 (13)	—
t(8;21)(q22;q22)	0 (0)	26 (8)	—
inv(16)(p13q22)	1 (1)	18 (5)	—
Abnormality involving 11q23	8 (12)	12 (4)	—
Loss of 5q	8 (12)	14 (4)	—
Loss of 7q	19 (28)	26 (8)	—
+8	9 (13)	40 (12)	—
Complex (≥3 aberrations)	22 (32)	39 (11)	—
Prior therapy			
Radiotherapy only	38 (43)	—	—
Any chemotherapy‡	51 (57)	—	—
Alkylating agents	24 (27)	—	—
Topoisomerase inhibitors	13 (15)	—	—
Antimetabolites	14 (16)	—	—
Tubulin inhibitors	13 (15)	—	—
Corticosteroids	15 (17)	—	—
Details not available	16 (18)	—	—

*French–American–British acute leukemia classification.

†Chromosomal abnormalities listed are the most common observed in AML and classification is not mutually exclusive. Percentages are based on the number of patients for whom cytogenetic investigation was successful.

‡Nineteen patients received both chemotherapy and radiotherapy. Alkylating agents: chlorambucil, dacarbazine, busulfan, mechlorethamine, procarbazine, ifosfamide, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), mitomycin C, any cisplatin derivative, melphalan, and cyclophosphamide. Topoisomerase inhibitors: mitoxantrone, adriamycin (doxorubicin), etoposide, epirubicin, idarubicin, and daunorubicin (bleomycin is also included in this group). Antimetabolites: methotrexate, azathioprine, 5-fluorouracil, cytosine arabinonucleoside, and hydroxyurea. Tubulin inhibitors: vincristine and vinblastine. Corticosteroids: prednisone and prednisolone (cyclosporin A is also included in this group). Patients are listed more than once if they received more than one type of therapy.

GST Genotype and Risk of t-AML by Type of Therapy. No association with t-AML and either *GSTM1* or *GSTT1* by therapy type was evident (Table 3). In contrast, the *GSTP1* codon 105 Val allele appears to confer a significantly increased risk of t-AML after chemotherapy (OR 2.66, 95% CI 1.39–5.09), but not after radiotherapy, compared with *de novo* AML (OR 1.01, 95% CI 0.50–2.07) (Table 3). Twenty-one of the 51 t-AML patients who had prior chemotherapy were exposed to at least one confirmed GSTP1 substrate, which is associated with an excess risk of t-AML with presence of at least one Val allele at *GSTP1* codon 105, compared with *de novo* AML patients (OR 4.34, 95% CI 1.43–13.20) (Table 4). However, a nonsignificant 2-fold in-

Table 2. GST genotype and the risk of developing t-AML or *de novo* AML

	Controls	*De novo* AML*			t-AML		
Variable	*n* (%)	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI
GSTM1†							
Carriers	523 (51)	188 (45)	1	—	40 (45)	1	—
Null	496 (49)	229 (55)	1.28	1.02–1.61	49 (55)	1.31	0.84–2.03
SNA	3	3			0		
GSTT1†							
Carriers	879 (86)	338 (81)	1	—	70 (79)	1	—
Null	140 (14)	79 (19)	1.47	1.08–1.99	19 (21)	1.79	1.04–3.10
SNA	3	3			0		
GSTP1‡							
Ile/Ile	497 (49)	202 (49)	1	—	33 (37)	1	—
Ile/Val	378 (37)	151 (36)	0.98	0.76–1.26	40 (45)	1.51	0.93–2.46
Val/Val	140 (14)	61 (15)	1.06	0.75–1.50	16 (18)	1.80	0.95–3.39
SNA	7	6			0		
GSTP1‡							
Ile/Ile	497 (49)	202 (49)	1	—	33 (37)	1	—
Ile/Val + Val/Val	518 (51)	212 (51)	1.00	0.80–1.26	56 (63)	1.58	1.01–2.49
SNA	7	6			0		

ORs and 95% CIs were estimated relative to controls for an unmatched analysis adjusted for age and sex. SNA, sample not amplifiable or not available.

*A similar analysis using the majority of the patients and controls described in this study has been published previously (31).

†Carriers of at least one intact allele are used as reference.

‡Isoleucine homozygotes (Ile/Ile) are used as reference.

creased risk of t-AML was still present in persons previously treated with other or unknown chemotherapeutic agents (OR 2.00, 95% CI 0.91–4.40) (Table 4).

Discussion

Our findings suggest that t-AML, but not *de novo* AML, is associated with a polymorphism in the gene that encodes GSTP1. Specifically, these data suggest that individuals either heterozygous (Ile/Val) or homozygous for Val at codon 105 of *GSTP1* were twice as likely as Ile homozygotes to develop t-AML after chemotherapy (OR 2.66, 95% CI 1.39–5.09), and greater than 4 times more likely if they had been exposed to a known GSTP1 substrate (OR 4.34, 95% CI 1.43–13.20). A statistically nonsignificant 2-fold increased risk of t-AML was still observed when individuals were not exposed to a known GSTP1 substrate, suggesting that there are other chemotherapeutic agents yet to be confirmed as substrates. Unlike *GSTP1*, the risks associated with *GSTT1* and *GSTM1* gene-deletion polymorphisms appear no different for t-AML and *de novo* AML, consistent with previous reports (15, 31, 47, 48).

The more frequent occurrence of abnormalities involving 11q23 or the loss of 5q or 7q among t-AML patients is consistent

Table 3. GST genotype and the risk of developing t-AML by type of previous therapy

		t-AML								
	De novo AML	*n* (%)	Total t-AML cases			Radiotherapy			Chemotherapy	
Variable	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI	
*GSTM1**										
Carriers	188 (45)	40 (45)	1	—	15 (39)	1	—	25 (49)	1	—
Null	229 (55)	49 (55)	0.99	0.62–1.60	23 (61)	1.25	0.61–2.60	26 (51)	0.85	0.48–1.53
SNA	3	0			0			0		
*GSTT1**										
Carriers	338 (81)	70 (79)	1	—	33 (87)	1	—	37 (73)	1	—
Null	79 (19)	19 (21)	1.19	0.67–2.13	5 (13)	0.66	0.26–1.84	14 (27)	1.61	0.83–3.14
SNA	3	0			0			0		
GSTP1†										
Ile/Ile	202 (49)	33 (37)	1	—	19 (50)	1	—	14 (27)	1	—
Ile/Val	151 (36)	40 (45)	1.87	1.11–3.17	12 (32)	0.94	0.42–2.12	28 (55)	2.87	1.45–5.67
Val/Val	61 (15)	16 (18)	1.67	0.84–3.30	7 (18)	1.16	0.43–3.13	9 (18)	2.17	0.89–5.29
SNA	6	0			0			0		
GSTP1†										
Ile/Ile	202 (49)	33 (37)	1	—	19 (50)	1	—	14 (27)	1	—
Ile/Val + Val/Val	212 (51)	56 (63)	1.81	1.11–2.94	19 (50)	1.01	0.50–2.07	37 (73)	2.66	1.39–5.09
SNA	6	0			0			0		

ORs and 95% CIs were estimated relative to *de novo* AML cases for an unmatched analysis adjusted for age and sex. SNA, sample not amplifiable.

*Carriers of at least one intact allele are used as reference.

†Isoleucine homozygotes (Ile/Ile) are used as reference.

Table 4. Comparison of t-AML cases previously treated with chemotherapy and *de novo* AML cases, by exposure to confirmed GSTP1 substrates or other chemotherapeutic agents and by genotype for *GSTP1*

		t-AML					
	De novo AML	Known GSTP1 substrate*			Other or unknown chemotherapeutic agents		
Variable	*n* (%)	*n* (%)	OR	95% CI	*n* (%)	OR	95% CI
GSTP1†							
Ile/Ile	202 (49)	4 (19)	1	—	10 (33)	1	—
Ile/Val	151 (36)	12 (57)	4.43	1.39–14.12	16 (53)	2.26	0.99–5.15
Val/Val	61 (15)	5 (24)	4.16	1.07–16.07	4 (13)	1.38	0.42–4.56
SNA	6	0			0		
GSTP1†							
Ile/Ile	202 (49)	4 (19)	1	—	10 (33)	1	—
Ile/Val + Val/Val	212 (51)	17 (81)	4.34	1.43–13.20	20 (67)	2.00	0.91–4.40
SNA	6	0			0		

ORs and 95% CIs were estimated relative to *de novo* AML cases for an unmatched analysis adjusted for age and sex. SNA, sample not amplifiable.

*Etoposide, chlorambucil, adriamycin, melphalan, cyclophosphamide, busulfan, ifosfamide, and any cisplatin derivative.

†Isoleucine homozygotes (Ile/Ile) are used as reference.

with prior exposure to DNA topoisomerase II inhibitors and alkylating agents, respectively (11, 49). Other than the relatively high percentage of patients with a t(15;17), the cytogenetic profile of these t-AML patients is similar to those reported in previous studies (11, 49, 50).

Five subjects had genetic alterations (assessed by cytogenetic analysis) affecting the chromosomal sites of *GSTM1, GSTT1,* or *GSTP1.* PCR was performed with genomic DNA extracted from leukemic bone marrow in these five individuals. Thus, it is possible that genotyping may be inaccurate because of loss of genetic material. However, three of these subjects showed only gains of genetic material at the involved sites, which is unlikely to affect genotyping. Deletions affecting 11q13 *(GSTP1*) were found in the remaining two individuals, both of whom were genotyped as Ile homozygotes at codon 105. The statistical significance of the results is not affected by assuming loss of a Val allele during leukemogenesis in these subjects and therefore considering them to be Ile/Val heterozygotes at the *GSTP1* codon 105 locus (data not shown). We also acknowledge the hypothetical possibility that point mutation at the *GSTP1* codon 105 locus during leukemic initiation and the subsequent selection of mutated clones during disease development may give rise to inaccurate genotyping if leukemic bone marrow is used as a source of DNA, as it is for some t-AML patients in this study. However, we feel it is unlikely that *GSTP1* mutations would be selected during tumor initiation or progression because this gene is neither a tumor suppressor nor a protooncogene. As such, we feel that inaccurate genotyping does not represent a significant problem in this study.

Forty-two of the 51 t-AML individuals with prior chemotherapy exposure were confirmed as United Kingdom Caucasians. The results of statistical analysis remained significant when individuals of unknown ethnic origin were excluded (OR 3.47, 95% CI 1.60–7.50). Twenty of the 21 t-AML patients with prior exposure to a known GSTP1 substrate were confirmed as United Kingdom Caucasians. The results of statistical analysis remained significant when the individual of unknown ethnic origin was excluded (OR 4.09, 95% CI 1.34–12.54).

Our data support the hypothesis that the *GSTP1* codon 105 polymorphism modulates the leukemogenic effect of certain chemotherapeutic agents; this is consistent with prior observations showing that this polymorphism alters protein function. Biochemical studies have demonstrated a lower thermal stability of GSTP1 Val-105 compared with GSTP1 Ile-105 (24, 25) and also lower conjugating activity in Val homozygotes compared with Ile homozygotes, with heterozygotes displaying intermediate activity (51). In contrast, the data reported in this study are not consistent with an allele dosage effect. When *GSTP1* was analyzed as a trichotamous variable, heterozygotes and Val homozygotes appeared to have a similarly increased risk of t-AML following chemotherapy (Ile/Val, OR 2.87, 95% CI 1.45–5.67; Val/Val, OR 2.17, 95% CI 0.89–5.29), which suggests a threshold effect rather than a dosage effect for t-AML susceptibility. However, the small number of Val homozygotes limits the interpretation of these data in this respect.

Individuals with at least one Val allele at codon 105 of *GSTP1* may have an underlying predisposition to cancers when exposure to environmentally derived or endogenously formed GSTP1 substrates is a risk factor (29). However, acute leukemia appears not to be one of these cancers because *GSTP1* codon 105 status is not a risk factor for *de novo* AML (31) (Table 2), providing evidence that the increased susceptibility to t-AML suggested in our study is specific to prior therapeutic exposure.

It remains possible that the population at risk of t-AML may be genetically biased because of a role for the *GSTP1* codon 105 polymorphism in modulating either susceptibility to primary cancer or survival after therapy. Indeed, the *GSTP1* codon 105 Val allele has been reported to be associated with a significantly increased risk of bladder and testicular cancer (29), and with a nonsignificantly increased risk of breast cancer (52). However, a similar distribution in *GSTP1* codon 105 genotype in healthy controls and t-AML patients with prior exposure to radiotherapy suggests that predisposition to primary cancer does not result in significant population bias. Individuals with Val at *GSTP1* codon 105 may respond better to chemotherapy given for their primary cancer because of lower GSTP1 activity and increased chemotherapy-induced cytotoxicity in target tumor tissue. Indeed, codon 105 Val homozygotes have a significantly better prognosis than codon 105 Ile homozygotes treated with cyclophosphamide and adriamycin (both GSTP1 substrates) for breast cancer (53). Improved prognosis and long-term survival after therapy would increase the prevalence of individuals with Val at codon 105 in the population at risk of t-AML, although, unfortunately, we are presently unable to determine the potential effect on our results.

Bone marrow is particularly sensitive to the toxic effects of chemotherapeutic alkylating agents, which include cyclophosphamide, chlorambucil, busulfan, and ifosfamide, among others (54). The reasons for this remain unclear, although low DNA repair activity and lack of GST α expression in bone marrow $CD34^+$ cells have been implicated (55, 56). Toxicity manifests

clinically as pancytopenia, and it is dose-limiting for several chemotherapeutic alkylating agents. One approach to overcome acute toxicity is to artificially protect the bone marrow by using gene transfer techniques. Presumably this approach would also protect against mutagenesis and reduce the risk of t-AML. Use of *GSTP1* as the transgene has proven successful in protecting human bone marrow stem cells and bone marrow in animal models against the toxic effects of chemotherapeutic alkylating agents (37–39). Our results highlight the importance of selecting the appropriate transgene variant to confer maximum protection and suggest codon 105 as a residue that may be artificially mutated to potentially generate variants with even higher catalytic activity.

In conclusion, our data suggest that individuals with at least one *GSTP1* codon 105 Val allele are at a significantly increased risk of developing t-AML, compared with Ile homozygotes, after exposure to such chemotherapeutics as cyclophosphamide, chlorambucil, adriamycin, etoposide, and cisplatin derivatives. These agents form the backbone of chemotherapeutic regimes for the treatment of numerous malignancies, including lymphatic, bladder, breast, ovarian, lung, and testicular cancer. If *GSTP1* codon 105 status can identify individuals at high risk of developing a second therapy-related cancer, then it may be possible to tailor chemotherapy to minimize leukemogenic potential. Of course, the primary aim of any cancer therapeutic regime is to cure the patient of the disease. However, with high cure frequency and long-term survival after treatment for such cancers as pediatric Hodgkin's disease (57) and pediatric acute lymphocytic leukemia (58), the threat of t-AML in later life must be considered. *GSTP1* codon 105 status may also be used to identify patients who may benefit from more intensive surveillance for t-AML after chemotherapy.

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