

Association between Survival after Treatment for Breast Cancer and Glutathione S-Transferase P1 Ile¹⁰⁵Val Polymorphism¹

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Abstract

A glutathione S-transferase (GST) P1 polymorphism results in an amino acid substitution, Ile¹⁰⁵Val; the Val-containing enzyme has reduced activity toward alkylating agents. Cancer patients with the variant enzyme may differ in removal of treatment agents and in outcomes of therapy. We evaluated survival according to *GSTP1* genotype among women ($n = 240$) treated for breast cancer. Women with the low-activity Val/Val genotype had better survival. Compared with Ile/Ile, hazard ratios for overall survival were 0.8 (95% confidence interval, 0.5–1.3) for Ile/Val and 0.3 (95% confidence interval, 0.1–1.0) for Val/Val (P for trend = 0.04). Inherited metabolic variability may influence treatment outcomes.

Introduction

Despite the favorable prognosis for many women treated for breast cancer, in some instances cancer will recur, presumably because some tumor cells survive primary therapy. Inherited variability in metabolism of therapeutic agents is expected to be responsible, in part, for individual differences in response to cancer treatment (1). Cyclophosphamide-based chemotherapy regimens and radiotherapy are widely used in the treatment of breast cancer. The reactive molecules responsible for cytotoxicity of these therapies are subject to enzymatic removal, and variability of cells in sensitivity to therapy could depend, in part, on the availability and activity of specific metabolizing enzymes. GST³ enzymes are an important cellular defense system that protects cells from chemical injury by catalyzing conjugation of reactive electrophilic molecules with glutathione. GSTs catalyze detoxification of alkylating agents used in chemotherapy and detoxification of products of reactive oxidation (2). The pi-class human GST, *GSTP1*, was shown to catalyze glutathione conjugation of reactive cyclophosphamide metabolites in *in vitro* assays (3). *GSTP1* is also thought to play a role in protection from oxidative damage. *GSTP1* is the major GST expressed consistently in both normal and tumor breast tissue (4). The *GSTP1* gene is polymorphic, with important differences in activity according to genotype. Single nucleotide substitutions at A³¹³G (5) and C³⁴¹T (6) result in amino acid changes Ile¹⁰⁵Val and Ala¹¹⁴Val, respectively. The *GSTP1* Ile¹⁰⁵Val substitution is located near the substrate binding site of the enzyme, and the variant is fairly common in Caucasians. For example, in a healthy population, 51% were homozygous for the common allele, *GSTP1*

Ile/Ile, 43% were heterozygous, for *GSTP1* Ile/Val, and 6% were homozygous for the variant allele, *GSTP1* Val/Val (5). The *GSTP1* Val¹¹⁴ variant is infrequent, with <15% of Caucasians genotyped having a *GSTP1* Val¹¹⁴ allele (6). Differences in specific activity between *GSTP1* enzymes containing Val compared with Ile at position 105 have been demonstrated with several classes of substrates. Catalytic efficiency with the chemotherapeutic drug thiotepa was demonstrated to be 2-fold lower for the *GSTP1* Val¹⁰⁵ variant compared with *GSTP1* Ile¹⁰⁵ (7). Because thiotepa and cyclophosphamide act through related alkylating intermediates, it is likely that the *GSTP1* Val¹⁰⁵ variant will also differ in activity toward cyclophosphamide. Similarly, *GSTP1* variants may also differ in detoxification of reactive oxidant damage, although this has not been assayed. We hypothesized that cancer patients with the *GSTP1* Val¹⁰⁵ variant genotype may respond differently to treatment because of altered activity in enzymatic removal of treatment agents and ultimately may have differences in survival. We investigated survival according to inherited *GSTP1* Ile¹⁰⁵Val genotype among women treated for breast cancer.

Subjects and Methods

Eligible Subjects. This study was conducted at the Arkansas Cancer Research Center and approved by the Institutional Review Board of the University of Arkansas for Medical Sciences, a research hospital. Women receiving chemotherapy or radiation as first course of therapy for incident, primary, invasive breast cancer at Arkansas Cancer Research Center from 1985 to 1996 were identified through the tumor registry. Women with a history of prior cancer were excluded. For each subject, age, race, and follow-up information were obtained from the registry. The registry actively conducts annual follow-up for each patient, contacting the physician or the patient, and maintains information on date last contacted, vital status, and recurrence status. Registry records were also used to obtain information on type of therapy received (*i.e.*, surgery, chemotherapy, radiation, or hormonal therapy) and dates of treatment. Registry and pathology records were reviewed for information on disease characteristics at time of diagnosis, including stage, positive nodes, and estrogen and progesterone receptor status.

Genotyping. Archived paraffin blocks from surgery were the source of tissue for genotyping. Only women with normal tissue available for genotyping were included in the study ($n = 240$). The majority (76.4%) of normal tissue specimens were normal lymph nodes; the remainder were skin or breast tissue. For each subject, 50- μ m sections were cut and placed in sterile tubes for DNA extraction. Tissue was deparaffinized, and DNA was extracted using a commercial kit (Qiagen). We detected the Ile¹⁰⁵Val polymorphism by a PCR-RFLP-based method that has been described previously (8). Restriction enzyme *B*SmaI (New England Biolabs, Hertfordshire, United Kingdom) was substituted for *Alw*26I, resulting in an equivalent digestion.

Statistical Analysis. Association between *GSTP1* Ile¹⁰⁵Val genotype and patient characteristics were assessed by χ^2 test and Fischer's exact test. Survival and recurrence in relation to genotype were evaluated using Kaplan-Meier survival function and Cox proportional hazards models. For overall survival analysis, time from diagnosis to death or last follow-up was calculated. Hazard ratios estimated from the Cox model represent relative risk of death among women heterozygous or homozygous for the variant *GSTP1*

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³ The abbreviations used are: GST, glutathione S-transferase; CI, confidence interval.

Table 1 Selected characteristics of 240 women treated for breast cancer, by GSTP1 Ile¹⁰⁵Val genotype

	All subjects (%)	GSTP1 codon 105 genotype		
		Ile/Ile (%)	Ile/Val (%)	Val/Val (%)
Total	240	110	107	23
Age at diagnosis				
≤39	26 (10.8)	13 (11.8)	10 (9.4)	3 (13.0)
40–49	82 (34.2)	35 (31.8)	36 (33.6)	11 (47.8)
50–59	71 (29.6)	36 (32.7)	32 (29.9)	3 (13.0)
60–69	42 (17.5)	19 (17.3)	18 (16.8)	5 (21.7)
70+	19 (7.9)	7 (6.4)	11 (10.3)	1 (4.4)
Race				
Caucasian	192 (80.0)	93 (84.6)	80 (74.8)	19 (82.6)
African-American	48 (20.0)	17 (15.5)	27 (25.2)	4 (17.4)
Stage				
I	65 (27.1)	32 (29.1)	27 (25.2)	6 (26.1)
II, node negative	48 (20.0)	19 (17.3)	23 (21.5)	6 (26.1)
II, node positive	73 (30.4)	38 (34.6)	29 (27.1)	6 (26.1)
III	41 (17.1)	18 (16.4)	19 (17.8)	4 (17.4)
IV	13 (5.4)	3 (2.7)	9 (8.4)	1 (4.4)
Estrogen receptor status				
Positive	147 (61.3)	62 (56.4)	71 (66.4)	14 (60.9)
Negative	93 (38.8)	48 (43.6)	36 (33.6)	9 (39.1)
Progesterone receptor status				
Positive	111 (46.3)	49 (44.6)	54 (50.5)	8 (34.8)
Negative	129 (53.8)	61 (55.5)	53 (49.5)	15 (65.2)

Val¹⁰⁵ allele compared with women homozygous for the more common GSTP1 Ile¹⁰⁵ allele. Hazard ratios were calculated from the Cox model first by univariate analysis and then from a multivariate model with adjustment for prognostic factors. In the adjusted model, stage and node status at diagnosis (categories as shown in Table 1), and age at diagnosis (four categories: <40, 40–49, 50–69, and ≥70) were included as stratifying variables, and race (Caucasian or African-American, excluding other or unknown) and estrogen and progesterone receptor status were included as covariates. Trend was evaluated using likelihood ratio tests comparing models with and without a variable representing the number of variant alleles (0, 1, and 2); reported *P*s for trend tests are two-sided. For analysis of disease-free survival, time from disease-free date to recurrence, death, or last follow-up was calculated, and adjusted hazard ratios were estimated from the Cox model, including prognostic factors as described for analysis of overall survival.

Results

Of the 240 subjects genotyped, 189 women had received chemotherapy (72 received both chemo- and radiotherapy and 117 received chemotherapy only) and 51 had received radiotherapy but no chemotherapy. Among women receiving chemotherapy, when specific chemotherapy agents were noted, the most commonly used agents were cyclophosphamide (received by 95%), 5-fluorouracil (80%), and Adriamycin (76%). Deaths of 71 women were recorded by the registry, with cancer as cause of death for 49, other causes for 6, and unknown causes for 16. Median follow-up among 169 women alive at last contact was 58 months. Disease recurrence was recorded for 69 women. Among women with recurrence and with information available on therapies received after recurrence, 60% received chemotherapy after recurrence, and 37% received radiotherapy.

Characteristics of the study population are shown in Table 1. The study population included more women <50 years of age at diagnosis, and more with stage at diagnosis above I than would be expected in an incident case group. The overrepresentation of younger age and higher stage was present among all breast cancer cases in the Arkansas Cancer Research Center tumor registry. Women with these characteristics seemed to have been more likely to be referred to this research hospital for treatment than other incident cases. The distribution of GSTP1 genotypes was 48% GSTP1 Ile/Ile, 42% GSTP1 Ile/Val, and 10% GSTP1 Val/Val among 192 Caucasian cases and 35% GSTP1 Ile/Ile, 56% GSTP1 Ile/Val, and 8% GSTP1 Val/Val among 48 African-American cases. Table 1 shows the distribution of demographic and pathological features according to genotype. Pathological features did not differ significantly by genotype.

The Kaplan-Meier function for survival by GSTP1 genotype is shown in Fig. 1. GSTP1 genotype was associated with overall survival in analysis by Cox proportional hazards model, adjusted for age, race, stage at diagnosis, and estrogen and progesterone receptor status. Hazard ratios are shown in Table 2. Compared with women with GSTP1 Ile/Ile genotype, there was a trend of better survival (*P* = 0.04) with increasing number of GSTP1 Val alleles. In the Kaplan-Meier plot, survival in the heterozygous Ile/Val group was very similar to the homozygous Ile/Ile women, and the hazard ratio from the univariate Cox model for the Ile/Val group was 1.0. How-

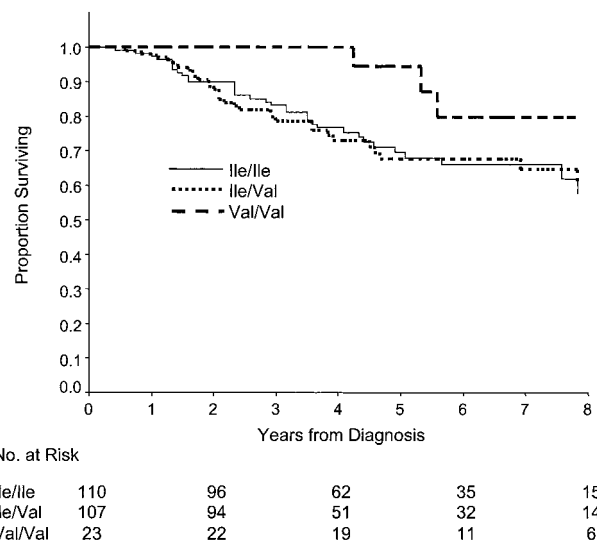


Fig. 1. Kaplan-Meier function for overall survival among women treated for breast cancer, by GSTP1 Ile¹⁰⁵Val genotype.

Table 2 Survival among 240 women treated for breast cancer, by GSTP1 Ile¹⁰⁵Val genotype

GSTP1 codon 105 genotype	No. of cases	Deaths	Hazard ratio ^a (95% CI)
Ile/Ile	110	35	1 Reference
Ile/Val	107	33	0.8 (0.5–1.3)
Val/Val	23	3	0.3 (0.1–1.0)

^a Hazard ratios from Cox proportional hazards model, adjusted for age, race, stage at diagnosis, node status, and estrogen and progesterone receptor status. Trend in hazard ratio associated with number of GSTP1 variant alleles, *P* = 0.04.

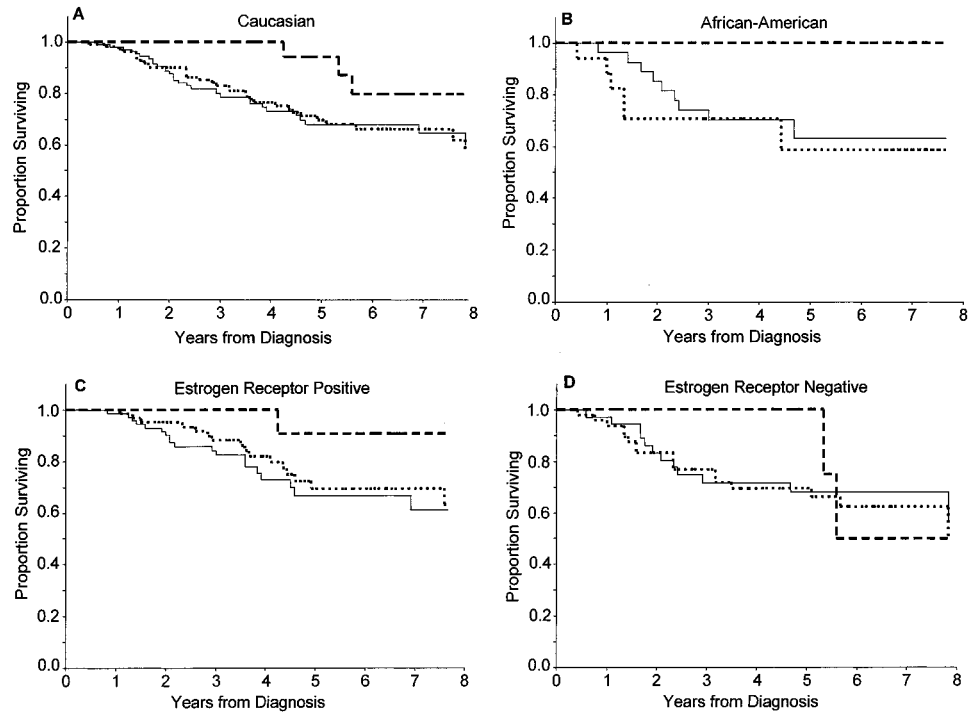


Fig. 2. Kaplan-Meier function for overall survival among women treated for breast cancer, by *GSTP1* *Ile*¹⁰⁵*Val* genotype, according to race and estrogen receptor status. —, *Ile/Ile*; ---, *Ile/Val*; ····, *Val/Val*. A, Caucasian, *n* = 93 *Ile/Ile*, *n* = 80 *Ile/Val*, *n* = 19 *Val/Val*. B, African American, *n* = 17 *Ile/Ile*, *n* = 27 *Ile/Val*, *n* = 4 *Val/Val*. C, estrogen receptor positive, *n* = 62 *Ile/Ile*, *n* = 71 *Ile/Val*, *n* = 14 *Val/Val*. D, estrogen receptor negative, *n* = 48 *Ile/Ile*, *n* = 36 *Ile/Val*, *n* = 9 *Val/Val*.

ever, there was confounding by stage at diagnosis. Nine of the 107 women in the *Ile/Val* group but only 3 of 110 in the *Ile/Ile* group were stage IV, so that after adjustment for stage, the hazard ratio changed to 0.8. When the analysis was limited to deaths that occurred within 3 years or within 5 years after diagnosis, adjusted hazard ratios for the *GSTP1* *Ile/Val* and *Val/Val* genotypes were similar to those in Table 2. When the analysis was restricted to women treated by chemotherapy, hazard ratios were essentially unchanged. Subgroup analyses were conducted by age and by estrogen receptor status (Fig. 2). Although there was little statistical power for assessment of survival differences by *GSTP1* within subgroups, on visual inspection of the Kaplan-Meier functions, it appears that the association between the homozygous *GSTP1* *Val*¹⁰⁵ variant genotype and better survival is present in each subgroup.

A second analysis was conducted considering disease-free survival, evaluating time from disease-free date to recurrence or death. Excluding 8 subjects described as "never disease free," for this analysis 82 subjects had recurred or died, and 141 were alive and free of recurrence at the end of follow-up. Hazard ratios were 1.0 (95% CI, 0.6–1.6) for the *GSTP1* *Ile/Val* group and 0.7 (95% CI, 0.3–1.8) for the *GSTP1* *Val/Val* group. In further analysis, we evaluated overall survival by *GSTP1* genotype after recurrence. Among women who had a recurrence, the time from recurrence to death was significantly longer, and hazard of death was less (*P* for trend = 0.05) for women with the *GSTP1* *Val* allele; hazard ratios were 0.8 (95% CI, 0.4–1.8) for *GSTP1* *Ile/Val* women and 0.2 (95% CI, 0.04–1.0) for the *GSTP1* *Val/Val* group.

Discussion

In this study, women with two inherited alleles for the *GSTP1* *Val* variant, which has lower specific activity toward alkylating agents, had better overall survival after treatment for breast cancer than women homozygous for the *GSTP1* *Ile* allele. The hazard of death among women homozygous for the variant allele was 30% of that for women homozygous for the common allele. This result is consistent with the hypothesis that therapy would be more successful among

patients with less activity in removal of chemotherapy agents because of the presence of the less active, variant form of *GSTP1*. The significant trend across genotypes was evidence of a gene-dose effect. Although the *GSTP1* genotype showed little association with time to recurrence, women with the *GSTP1* *Val/Val* genotypes had longer survival after recurrence than those with *GSTP1* *Ile/Ile*. To our knowledge, there is only one prior published study of *GSTP1* genotype and response to treatment for cancer. Among children with acute lymphoblastic leukemia (9), patients with *GSTP1* *Val/Val* genotype had an almost 3-fold reduction in risk of relapse. A role of chance in the present study should be considered. Our conclusion of a difference in survival relies largely on the experience of women with the *Val/Val* genotype, a group of only 23 subjects. However, the survival difference was significant at the *P* = 0.05 level, and the reduction in hazard was strong; therefore, this finding, if replicated in future studies, would be of clinical significance.

Inherited differences in *GSTP1* activity in removal of chemotherapy agents, and specifically of cyclophosphamide, is a plausible explanation for the association with survival observed in the present study, particularly in light of laboratory evidence that the *GSTP1* enzyme exhibits specific activity in glutathione conjugation of cyclophosphamide intermediates (3). When survival analysis in the present study was restricted to subjects who received chemotherapy, hazard ratios were essentially the same as those from the analysis including the total study population. *GSTP1* activity in detoxification of base propenals (10), products of hydroxyl radical reaction with DNA, may implicate *GSTP1* in protection from radiation damage; however, it is unclear whether base propenals are of critical importance in the context of overall damage to cells from reactive oxidation (11). Only 51 subjects in our study received radiation therapy only, and most of these were stage I patients who were alive and free of recurrence at the end of observation. Therefore, it was not possible to analyze survival according to *GSTP1* status among the radiotherapy-only group or to evaluate statistically whether the association between *GSTP1* genotype and survival differed by type of treatment.

GSTP1 expression in tumor tissue as measured by immunohisto-

chemistry predicts poorer prognosis for cancers of several sites, *e.g.*, ovary (12, 13). Studies of GSTP1 enzyme expression and prognosis in women treated for breast cancer (14–18), however, have not provided consistent evidence of a relationship. Our finding of a survival difference in women with breast cancer according to host constitutive *GSTP1* genotype is biologically plausible, even in the absence of a relationship between breast tumor GSTP1 expression and prognosis. Among individuals with similar levels of GSTP1 expression in tumor, enzyme catalytic activity would be expected to vary according to presence of variant *GSTP1* genotype. Furthermore, GSTP1 is expressed in many other tissues, including liver and RBCs, and the *GSTP1* genotype may modify the effective tumor dose of chemotherapy by altering systemic drug metabolism.

The association between *GSTP1* genotype and survival that we observed remained after adjustment for age, stage at diagnosis, node status, race, and hormone receptor status, indicating that the association was not the result of racial variation, nor was the association attributable to relationships between *GSTP1* genotype and one of these prognostic factors. In the present study, information on tumor grade, p53 expression, HER2/neu expression, and multidrug resistance protein expression was unavailable; therefore, we cannot exclude a relationship between one of these pathological features and *GSTP1* genotype as a mechanism for the association between *GSTP1* and survival. For example, Nedelcheva *et al.* (19) reported that loss of heterozygosity at the *p53* locus in breast cancer was more frequent among women with a *GSTP1 Val¹⁰⁵* variant allele. However, the relationship between *GSTP1* genotype and one of these unmeasured prognostic factors would have to be quite strong to account for the observed association between *GSTP1* and survival. Future research on metabolic polymorphisms and breast cancer prognosis should take into account molecular prognostic factors to consider whether genetic variation of the host acts independently, or through a causal pathway with other prognostic markers as intermediates, in predicting therapeutic response and survival.

In summary, our data indicate that host constitutional metabolic variability may greatly impact the efficacy of treatment for breast cancer. Although the role of genetic variability in metabolic enzymes has been studied extensively in relation to chemical carcinogenesis and cancer risk, there has been little attention to the impact of pharmacogenetics on response to treatment for cancer, particularly for breast cancer. Further research in this field could contribute to more individualized cancer treatment strategies in the future.

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