

## Polymorphisms in manganese superoxide dismutase, myeloperoxidase and glutathione-S-transferase and survival after treatment for metastatic breast cancer

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**Abstract** Treatments for metastatic breast cancer (MBC) are primarily palliative with variable efficacy and outcomes may be influenced by individual differences in drug metabolism. In this study, we examined the association of single nucleotide polymorphisms (SNPs) in genes involved in drug metabolism with progression free survival (PFS) and breast cancer specific survival (BCSS) in 95 patients with MBC that received high dose chemotherapy (HDC) with autologous stem cell transplantation (ASCT). SNPs in the *SOD2* (SOD2-01, Val16Ala), *MPO* (MPO-02, -463 promoter variant) and *GSTP1* [GSTP1-01 (Ile105Val), GSTP1-02 (Ala114Val)] genes were examined in DNA isolated from cryopreserved blood products using genotyping assays. Survival was analysed using Cox proportional hazard models and Kaplan–Meier estimates. Patients with the SOD2-01 (TT) genotype had increased risk of disease progression [hazard ratio (HR): 2.52; 95% confidence interval (CI), 1.31–4.85] and breast cancer specific death (HR: 1.92; 95% CI: 1.03–3.57). Risks were increased for patients with both SOD2-01 (TT) and GSTP1-01 (GG or AG) genotypes (HR for disease progression: 2.57, 95% CI: 1.32–5.00 and HR for breast cancer specific death: 2.27; 95% CI: 1.18–4.34). In multivariable analysis, the combined genotype group of SOD2-01 and GSTP1-01 was an independent predictor of PFS and BCSS. HRs progressively increased with increasing number of genotypes associated with worse survival, with  $p_{(\text{trend})}$  of

0.005 and 0.006 for PFS and BCSS, respectively. These results suggest that SNPs in genes involved in drug metabolism may influence survival outcome for patients with MBC receiving HDC and ASCT.

**Keywords** Drug metabolism · Metastatic breast cancer · Polymorphisms · Survival

### Introduction

Chemotherapeutic drug efficacy is complex and can be influenced by cellular detoxification mechanisms involving metabolic and drug transport pathways [1]. The products of the *SOD2*, *MPO* and *GSTP1* genes are involved in the metabolism of some of these drugs and a number of studies have shown that single nucleotide polymorphisms (SNPs) within these genes can alter protein expression and/or function. Some studies have shown that these SNPs may contribute to cancer risk [2, 3] and patient survival [4–6] following chemotherapy.

Chemotherapy drugs including anthracyclines (doxorubicin, epirubicin and daunorubicin), alkylating agents (cyclophosphamide and thiotepa) and platinum drugs (cisplatin, carboplatin and oxaliplatin) can generate high levels of reactive oxygen species (ROS) [7]. ROS such as hydroxyl radicals, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and superoxide anions can cause cellular damage to DNA, lipids and proteins resulting in cell death or inhibition of cell proliferation [8, 9]. Although taxanes (paclitaxel and docetaxel), vinca alkaloids (vincristine and vinblastine) and antifolates primarily block cell cycle progression, they can also increase cellular levels of ROS [7, 10–12].

The product of the *SOD2* gene, manganese superoxide dismutase (MnSOD), is a mitochondrial enzyme that

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catalyzes the formation of  $H_2O_2$  from superoxide radicals generated by chemotherapeutics. A SNP in the *SOD2* gene, SOD2-01 (Val16Ala, rs4880), is a C (alanine) to T (valine) substitution in the mitochondria targeting sequence that causes defective localization of the enzyme to the inner mitochondrial membrane. In vitro, cancer cells transfected with the C (Ala) variant have fourfold higher levels and increased activity of the MnSOD protein than cells transfected with the T (Val) variant [13]. Studies have shown a significant association of this polymorphism with survival outcome and/or risk in breast [5, 14, 15], colorectal [16], prostate [17] and bladder [18] cancers.

The  $H_2O_2$  generated by MnSOD can be further converted to highly damaging hydroxyl radicals via myeloperoxidase (MPO), the product of the *MPO* gene [19].

MPO is a metabolic/oxidative lysosomal enzyme found in neutrophils and monocytes [20]. MPO is involved in the production of oxidative hypochlorous acid from  $H_2O_2$  during infection and in the metabolic activation of a number of procarcinogens, including benzo( $\alpha$ )pyrene, 4-aminobiphenyl and the arylamines found in tobacco smoke [21]. The SNP, MPO-02, is a single base substitution of G to A at position -463 of the promoter region (rs2333227) of the *MPO* gene. The MPO-02 A allele alters a SP1-binding site resulting in decreased transcription of the gene and  $\sim 25$  times lower activity [22]. Studies have reported an association of this SNP with lung [23, 24], bladder [18] and esophageal [25] cancer risk and with breast cancer survival [5].

A number of glutathione-associated pathways protect cells against agents which generate oxidative stress [26]. The glutathione-S-transferase family of phase II detoxification isozymes is comprised of at least 16 genes and plays major roles in protecting cells from oxidative stress and in the detoxification of free radicals generated by drugs and ionizing radiation [1]. Substrates of the GSTP1 isozyme include carboplatin, cisplatin, anthracyclines and various alkylating agents [27]. The expression of GSTP1 is associated with drug resistance in a number of cancers [28, 29]. Two SNPs in *GSTP1* alter the substrate binding site of the enzyme and decrease its activity for some drugs [30–32]. GSTP1-01 (GSTP1\*B, rs1695) is an A to G transition that results in an isoleucine to valine change at codon 105 (SNP region Exon 5 - 24A > G). GSTP1-02 (rs1138272) is a C to T transition that results in an alanine to valine change at codon 114 (SNP region: Exon 6 + 5C > T). Several studies have shown an association of the GSTP1-01 G (Val) allele with increased disease-free survival for ovarian and breast cancer [4, 6, 33]. Improved survival outcomes for patients with multiple myeloma and esophageal cancer were associated with the A (Ile) allele which has greater enzyme activity [34, 35].

We hypothesized that the functional polymorphisms, SOD2-01, MPO-02, GSTP1-01 and GSTP1-02, may

modulate the levels of ROS and therefore influence the efficacy of chemotherapy treatment. Further, combinations of polymorphisms in these genes may substantially affect the cytotoxicity of these drugs and influence survival outcome. Therefore, we examined the association of these SNPs with survival outcome in 95 patients with stage IV metastatic breast cancer (MBC) treated with high dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT).

## Materials and methods

### Patient population

Patient characteristics and HDC treatment regimens are listed in Table 1. There were 134 patients enrolled in one of five clinical trials of HDC with ASCT at SRH (Sudbury Regional Hospital) between 1992 and 1997. Within this group, there were 102 stage IV patients who received HDC and ASCT and DNA was available from 95 of these patients. Estrogen and progesterone receptor status were not available for all patients (not done or inconclusive). Information on tissue human epidermal growth factor receptor 2 (HER-2) status was unavailable.

Patients received 2–4 cycles of mobilization chemotherapy consisting of 5-fluorouracil, adriamycin or epirubicin and cyclophosphamide, or 5-fluorouracil, epirubicin and mitoxantrone. HDC treatments and cumulative dosages are described in Table 1. Five patients received a second HDC of cyclophosphamide, mitoxantrone and either carboplatin ( $n = 4$ ) or vinblastine ( $n = 1$ ). One patient received a second HDC of cyclophosphamide and thiotepa. The clinical trials and study were approved by the Research Ethics Board, Sudbury Regional Hospital, Sudbury, ON and informed signed consent was obtained from all patients.

### Analysis of SNPs

DNA was extracted from cryopreserved, apheresis blood product, peripheral blood or bone marrow samples using the DNA Blood MiniKit (Qiagen, Mississauga, ON, Canada) following the manufacturer's protocol.

Similar to a previous study, a candidate approach was used to select for non-synonymous SNPs with moderate frequency in genes previously reported to be associated with chemotherapeutic sensitivity, cancer risk, progression or survival [36]. The National Cancer Institute SNP500Cancer database (<http://snp500cancer.nci.nih.gov/snplist.cfm>) provided information on SNPs including target sequence, frequency estimates and referenced, validated

**Table 1** Clinical characteristics and treatment regimens of metastatic breast cancer patients

Clinical characteristic	Number of patients	
	Number	%
Total (stage IV)	95	
Age		
<40	23	24
40–49	49	52
50–59	23	24
ER ( <i>n</i> = 84)		
Negative	35	42
Positive	49	58
PR ( <i>n</i> = 80)		
Negative	41	51
Positive	39	49
Number of metastatic sites		
UD, 1	54	57
≥2	41	43
Metastatic sites <sup>a</sup>		
Bone	53	56
Lung	32	34
Lymph node	26	27
Liver	14	15
Other	22	23
HDC regimen		
Mitox, Cyclo, Vin	27	28
Mitox, Cyclo, Carbo	21	22
Mitox, Cyclo, Paclitaxel	42	44
Thiotepa, Cyclo, Carbo	4	4
Mitox, Cyclo	1	1

ER estrogen receptor, PR progesterone receptor, UD unassessable disease, HDC high dose chemotherapy, Mitox mitoxantrone (cumulative dose: 70 mg/m<sup>2</sup>), Cyclo cyclophosphamide [cumulative dose (CD): 6 g/m<sup>2</sup>], Vin vinblastine (CD: 12 mg/m<sup>2</sup>), Carbo carboplatin (CD: 800 mg/m<sup>2</sup>), paclitaxel (CD: 250–400 mg/m<sup>2</sup>), thiotepa (CD: 500 mg/m<sup>2</sup>)

<sup>a</sup> Number determined at enrolment to study, includes metastasis to more than one site

5'-nuclease assays (TaqMan) using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The TaqMan assay consists of two primers for PCR amplification of the sequence of interest and two allele-specific fluorescent probes. SNPs, primer and probe sequences are: SOD2-01, primers: 5'- GCTGT GCTTTCTCGTCTTTCAG-3' and 5'-CTGCCTGGAGCCC AGATAC-3', allele specific probes: 5'-FAM-CCAAAACC GGAGCC-3'(MGB) and 5'-VIC-CCAAAGCCGGAGCC-3'(MGB); MPO-02, primers: 5'-TCTTGGGCTGGTAGT GC-3' and 5'-GTATTTTATAGTAGATACAGGGTTTCA-3',

allele specific probes: 5'-FAM-AGGCTGAGGCAGGTG GAT-3'(TAM), 5'-VIC-TGAGGCCGGTGGATCA CT-3' (TAMRA); GSTP1-01, primers: 5'-CCTGGTGGACATG GTGAATGAC-3' and 5'-TGGTGCAGATGCTCACATA GTTG-3', allele specific probes: 5'-FAM-CTGCAAATAC GTCTCC-3' (MGB) and 5'-VIC-CTGCAAATACATCT CC-3' (MGB) and, GSTP1-02, primers: 5'- AGTA GGA TGATACATGGTGGTGTCT-3' and 5'- GGCAGTGCCT TCACATAGTCAT-3' allele specific probes: 5'-FAM-CTTGCCACCTCCT-3' (MGB) and 5'-VIC- CTTGCC GCCTCCT-3' (MGB). For quality control purposes, genotyping of 70% of random samples for each SNP was repeated at least two times and only concordant results are reported. Assignment of genotypes was performed independently by two investigators blinded to the survival endpoints.

### Statistical methods

For each SNP, median survival was calculated for each genotype and then for combinations of genotypes. Survival curves were generated using the Kaplan–Meier product limit estimate of the survivorship function. Fisher's exact test (significant at  $p < 0.05$ ) was used to evaluate whether patient and tumour characteristics (age group, ER status, PR status, presence of liver metastases, bone metastases and number of metastases) were associated with each genotype or combined genotypes for each SNP. Progression free survival (PFS) was defined as the time (months) from study registration until documented progression of metastatic disease or censorship (not progressed during follow-up time period). Breast cancer specific survival (BCSS) was defined as the time (months) from study registration until death from metastatic disease or censorship (alive at the end of the follow-up time period). Survival information was collected from clinical research records.

Equality of survivorship functions was assessed using the log-rank test. The Cox proportional hazard regression model defined hazard ratios (HR) and 95% confidence intervals (CI). Multivariable analysis by proportional hazards regression models used variables that were identified as significant in univariate analysis for both BCSS and PFS and that were available for the full data set. The estimates from these models provided HR and 95% CI adjusted for all variables in the model. Tests for trend were conducted by calculating  $p$ -values for the coefficient in proportional hazards regression models with the variable of interest coded as an ordinal variable.

Statistical analysis was done using Stata Version 8.0 (Stata Corporation, College Station, TX, USA).

## Results

### Patient characteristics

The median age at study entry was 45 years (range 19–56). During the follow-up period (median 10.4 months, range 1.6–111.7 months), disease progression occurred in 91 patients (96%) and 91 patients (96%) died due to MBC; four patients remained alive. The time at risk for disease progression ranged from 1.6 to 111.7 months. The time at risk for death ranged from 4.8 to 111.7 months. For the total group ( $n = 95$ ), the median PFS was 10.4 months and the median BCSS was 22.4 months.

The characteristics of the patient group are shown in Table 1. There was no association of SNP genotypes with ER status, PR status, liver, lung, lymph node and “other” metastases or number of metastatic sites. The presence of bone metastases was significantly associated with GSTP1-01 AA ( $p = 0.05$  for comparison among GSTP1-01 genotypes and  $p = 0.02$  for comparison between the GSTP1-01 AA and AG + GG genotypes).

Patients were assigned to four major groups based on differences in treatment regimens (the fifth group contained only one patient) (Table 1). There were no significant differences in PFS and BCSS (Kaplan–Meier survival and log rank analysis of survivorship function) between the major treatment groups, between patients receiving 1 or 2 HDC treatments, or for patients treated with or without carboplatin (data not shown).

In univariate analysis of patients with known hormone receptor status, there were significant differences for PFS [( $p$  determined from  $\chi^2(1) = 0.02$ )] and BCSS ( $p_{\chi^2(1)} = 0.005$ ) by estrogen receptor status ( $n = 84$ ) but not by progesterone receptor status ( $n = 80$ , PFS:  $p_{\chi^2(1)} = 0.27$ , BCSS:  $p_{\chi^2(1)} = 0.09$ ). Survival differences were significant for patients with metastatic site(s) that included liver ( $n = 14$ , PFS:  $p_{\chi^2(1)} < 0.001$ ; BCSS:  $p_{\chi^2(1)} < 0.001$ ). Patients with site(s) of metastases that included bone had significantly longer survival than any other metastatic sites ( $n = 53$ , PFS:  $p_{\chi^2(1)} = 0.03$ ; BCSS:  $p_{\chi^2(1)} = 0.02$ ).

Patients with more than one metastatic site ( $n = 41$ ) had significantly decreased PFS ( $p_{\chi^2(1)} = 0.002$ ) but not BCSS ( $p_{\chi^2(1)} = 0.15$ ) compared to patients with unassessable disease (UD) or one metastatic site.

### Breast cancer specific survival and progression free survival

The genotypic frequencies for each SNP are shown in Table 2. These frequencies are similar to those previously reported in the National Cancer Institute, SNP500Cancer Database [37]. The association of genotypes with PFS and

BCSS was analysed separately and for combined heterozygous and homozygous genotypes (Table 2). Median survival (PFS and BCSS) was decreased for patients with genotypes SOD2-01 TT, MPO-02 GG + AG, GSTP1-01 GG + AG or GSTP1-02 TT + CT. Patients with the SOD2-01 TT genotype had significantly increased risk of disease progression and death compared to patients with the SOD2-01 CC genotype. For patients with these genotypes (TT versus CC), the median PFS was 8.8 months vs. 11.6 months (HR: 2.52; 95% CI: 1.31–4.85) and the median BCSS was 18.6 months vs. 28.6 months (HR: 1.92; 95% CI: 1.03–3.57).

The GSTP1-01 G and GSTP1-02 T alleles were also associated with a decrease (non-significant) in PFS and BCSS. The data was suggestive of an association with increased risk for disease progression or breast cancer specific death and increasing number of the MPO-02 G alleles, although only four patients had the AA genotype limiting interpretation.

Differences in survival were greater when combinations of genotypes were considered (Table 3). The risk of breast cancer-specific death was significantly increased for patients with both the GSTP1-01 GG + AG and GSTP1-02 TT + CT genotypes (HR: 1.79; 95% CI: 1.00–3.22). Patients with combined SOD2-01 TT and GSTP1-01 GG + AG genotypes were also at significantly increased risk for disease progression (HR: 2.57; 95% CI: 1.32–5.00) and breast cancer specific death (HR: 2.27; 95% CI: 1.18–4.34). Although not significant, the results were suggestive of an increased risk of disease progression for patients with the combined GSTP1-01 GG + AG and GSTP1-02 TT + CT genotypes and for disease progression and breast cancer-specific death for patients with the combined (SOD2-01 TT and GSTP1-02 TT + CT) or (SOD2-01 TT and MPO-02 GG + AG) genotypes.

In a Cox proportional hazards model, that adjusted for age, bone and liver metastases (which were significantly associated with both PFS and BCSS in univariate analysis), the combined group of SOD2-01 and GSTP1-01 genotypes was an independent predictor for both PFS and BCSS. Metastatic sites that included bone independently predicted better PFS and BCSS (Table 4).

A subgroup of patients ( $n = 61$ ) was further examined in a Cox proportional hazards model adjusted for age, bone and liver metastases to determine the additional contribution to risk of GSTP1-01 in a model that contained SOD2-01. (Patients having MPO-02 GG + AG and/or GSTP1-02 TT + CT genotypes were excluded from the model.) Patients with the SOD2-01 TT genotype but not the GSTP1-01 GG + AG genotype ( $n = 11$ ) had a HR of 2.72 (95% CI: 1.28–5.79) for PFS and a HR of 1.85 (95% CI: 0.90–3.80) for BCSS [the referent patient group had SOD2-01 CC + CT and GSTP1-01 AA genotypes ( $n = 36$ )]. The

**Table 2** SNPs and progression free survival and breast cancer specific survival

Genotype	Number	Median PFS (months)	$p^a$	Age-adjusted HR (95% CI)	Median BCSS (months)	$p^a$	Age-adjusted HR (95% CI)
<b>SOD2-01</b>							
CC	20	11.6		1.0 Reference	28.6		1.0 Reference
CT	50	10.4	0.01	1.43 (0.82–2.49)	22.4	0.10	1.38 (0.80–2.39)
TT	25	8.8		<b>2.52 (1.31–4.85)</b>	18.6		<b>1.92 (1.03–3.57)</b>
CC + CT	70	10.7		1.0 Reference	25.1		1.0 Reference
TT	25	8.8	0.006	<b>1.93 (1.18–3.16)</b>	18.6	0.07	1.53 (0.96–2.44)
<b>MPO-02</b>							
AA	4	15.1		1.0 Reference	39.7		1.0 Reference
AG	34	10.7	0.6	1.71 (0.60–4.85)	22.9	0.4	2.05 (0.72–5.83)
GG	57	9.8		1.65 (0.59–4.60)	19.9		1.86 (0.67–5.17)
AA	4	15.1		1.0 Reference	39.7		1.0 Reference
GG + AG	91	9.8	0.3	1.67 (0.61–4.59)	21.4	0.2	1.92 (0.70–5.27)
<b>GSTP1-01</b>							
AA	47	11.0		1.0 Reference	25.7		1.0 Reference
AG	38	9.8	0.2	1.41 (0.91–2.19)	19.2	0.2	1.37 (0.88–2.13)
GG	10	8.8		1.51 (0.75–3.03)	17.2		1.51 (0.75–3.02)
AA	47	11.0		1.0 Reference	25.7		1.0 Reference
GG + AG	48	9.3	0.08	1.43 (0.94–2.17)	18.6	0.1	1.40 (0.92–2.12)
<b>GSTP1-02</b>							
CC	79	10.7		1.0 Reference	24.8		1.0 Reference
CT	14	8.8	0.4	1.40 (0.78–2.52)	18.5	0.2	1.62 (0.90–2.91)
TT	2	8.3		2.04 (0.49–8.49)	14.7		2.03 (0.49–8.43)
CC	79	10.7	0.3	1.0 Reference	24.8	0.09	1.0 Reference
TT + CT	16	8.8		1.46 (0.83–2.54)	18.5		1.66 (0.95–2.90)

<sup>a</sup> Log-rank for comparison between Kaplan-Meier survival curves were derived from comparisons among all three genotypes or between two groups in the combined genotype model

risk for disease progression and breast cancer death increased for patients with both SOD2-01 TT and GSTP1-01 GG + AG genotypes [(HR of 2.89 (95% CI: 1.42–5.94,  $p_{\text{trend}} = 0.002$ ) for PFS and HR of 2.17 (95% CI: 1.11–4.21,  $p_{\text{trend}} = 0.013$ ) for BCSS] suggesting an increased contribution to risk of 6 and 17% for PFS and BCSS, respectively, by adding GSTP1-01 to a model that contained SOD2-01.

#### Increasing number of “risk” genotypes and survival

In this study, the genotypes SOD2-01 TT, MPO-02 GG + AG, GSTP1-01 GG + AG and GSTP1-02 TT + CT were associated with worse survival and therefore we designated them as “risk” genotypes.

The association of an increasing number of these “risk” genotypes with survival was examined (Table 5). The reference group was defined to include patients with 0 or 1 “risk” genotypes since only two patients had 0 “risk” genotypes (median PFS 19.4 months and median BCSS 36.0 months). The risk for disease progression and breast cancer specific death increased with increasing number of

“risk” genotypes with the  $p_{(\text{trend})} = 0.005$  for PFS and the  $p_{(\text{trend})} = 0.006$  for BCSS. Patients with  $\geq 3$  “risk” genotypes (there were only two patients with all 4 “risk” genotypes) had significantly increased risk of disease progression (HR: 2.09; 95% CI: 1.24–3.49) and death (HR: 2.20; 95% CI: 1.31–3.70). In a separate analysis, comparison to the 0 + 1 “risk” group showed that patients with all 4 “risk” genotypes ( $n = 2$ ) had the greatest risk for PFS [HR: 3.63 (0.81–16.28)] and BCSS [HR: 2.31 (0.53–10.39)]. Kaplan Meier survival curves for the 0 + 1, 2 and 3 + 4 “risk” genotypes are shown in Fig. 1.

#### Discussion

In this study, we evaluated the association of SNPs in genes that are involved in the metabolism of drugs with survival outcome in patients with MBC undergoing HDC and ASCT. The results indicate that survival (median PFS and BCSS) was significantly decreased for patients with the SOD2-01 TT genotype. Although not significant, decreases in median PFS and BCSS were observed for the MPO-02 GG + AG, GSTP1-01 GG + AG and GSTP1-02 TT + CT

**Table 3** Genotype combinations and survival

Combined genotypes		<i>n</i>	Median PFS months	<i>p</i> <sup>a</sup>	Age-adjusted HR (95% CI)	Median BCSS months	<i>p</i> <sup>a</sup>	Age-adjusted HR (95% CI)
<b>GSTP1-01</b>	<b>GSTP1-02</b>							
AA	CC	47	11.0		1.0 Reference	25.7		1.0 Reference
GG + AG	AA	32	10.5	0.2	1.35 (0.84–2.16)	20.0	0.14	1.25 (0.78–2.00)
GG + AG	TT + CT	16	8.8		1.61 (0.90–2.89)	18.5		<b>1.79 (1.00–3.22)</b>
<b>SOD2-01</b>	<b>GSTP1-01</b>							
CC + CT	AA	36	12.8		1.0 Reference	25.7		1.0 Reference
TT CC + CT	AA GG + AG	45	9.8	0.01	1.57 (0.99–2.48)	22.4	0.03	1.33 (0.85–2.10)
TT	GG + AG	14	8.8		<b>2.57 (1.32–5.00)</b>	17.2		<b>2.27 (1.18–4.34)</b>
<b>SOD2-01</b>	<b>GSTP1-02</b>							
CC + CT	CC	56	12.4		1.0 Reference	25.7		1.0 Reference
TT CC + CT	CC TT + CT	37	9.1	0.01	<b>1.89 (1.21–2.96)</b>	17.7	0.03	<b>1.74 (1.13–2.70)</b>
TT	TT + CT	2	8.3		3.56 (0.79–15.96)	18.6		2.21 (0.51–9.61)
<b>SOD2-01</b>	<b>MPO-02</b>							
CC + CT	AA	2	14.2		1.0 Reference	19.9		1.0 Reference
CC + CT TT	GG + AG	70	10.7	0.003	1.50 (0.37–6.19)	25.7	0.02	1.21 (0.29–4.96)
TT	GG + AG	23	8.3		3.49 (0.80–15.3)	17.4		2.43 (0.56–10.5)

<sup>a</sup> Indicates log-rank for comparison among Kaplan Meier survival curves. Combinations of genotypes are reported only for those associated with risk

**Table 4** Multivariable analysis

Variable	PFS HR <sup>a</sup> (95% CI)	BCSS HR <sup>a</sup> (95% CI)
Bone metastases	<b>0.65 (0.42–0.99)</b>	<b>0.60 (0.39–0.92)</b>
Liver metastases	<b>2.76 (1.54–4.92)</b>	<b>2.70 (1.52–4.80)</b>
SOD2-01 or GSTP1-01	<b>1.61 (1.01–2.55)</b>	1.25 (0.79–1.97)
SOD2-01 and GSTP1-01	<b>2.41 (1.23–4.72)</b>	<b>2.05 (1.07–3.94)</b>

<sup>a</sup> Adjusted for all variables in model including age

genotypes. Patients with combinations and increasing numbers of these genotypes were at increased risk for disease progression and breast cancer specific death. These results suggest that the efficacy of chemotherapy treatment may be influenced by SNPs that alter oxidative stress levels and drug detoxification.

Patients with the SOD2-01 TT genotype were at significantly increased risk of disease progression and breast cancer specific death following chemotherapy. Previous studies have shown that the SOD2-01 TT genotype results in decreased enzyme levels and activity [13]. Therefore, chemotherapy may be more effective as a result of increased DNA damage for patients with the SOD2-01 CC genotype and the more active enzyme.

Few studies have examined the association of SOD2-01 with survival outcome of breast and other cancers. Ambrosone et al. reported an association of the SOD2-01 CC genotype (non-significant) and/or the MPO-02 GG genotype (significant) with decreased risk of death following

standard treatment of women with primary breast cancer [5]. They suggested that MPO may be able to augment the effect of MnSOD in the generation of ROS. However, in our study, the SOD2-01 TT genotype was significantly associated with increased risk of disease progression and death and although results were suggestive of an association of the MPO-02 AA genotype with increased survival, they were not significant. Although it is not possible to draw any conclusions from these results (there were only four patients with the AA genotype), higher adduct levels have been observed in breast tissue from women with the A allele of MPO-02 [38] providing biological support for these results. The A allele has been shown to result in decreased transcription and enzyme levels [22]. Larger studies will be required to further examine the influence of MPO-02 with survival outcome.

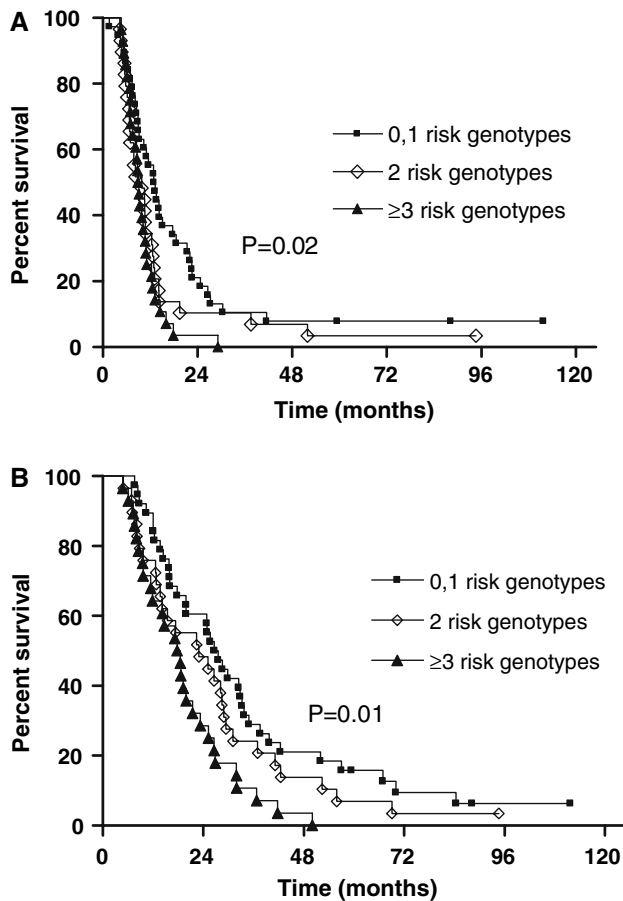
Although the increased risk of disease progression and breast cancer specific death appears to be primarily driven by the SOD2-01 TT genotype, risks further increased when we included GSTP1-01 in analysis. There was a significant risk of both disease progression and breast cancer specific death for women with the SOD2-01 (TT) and GSTP1-01 (GG + AG) genotypes with the risk of disease progression increasing by 6% and the risk of breast cancer specific death increasing by 17% compared to women with only the SOD2-01 TT “risk” genotype.

GSTP1 is the major GST enzyme involved in drug detoxification and in the reduction of free radicals generated by drugs and radiation. In this study, increasing

**Table 5** Increasing number of “risk” genotypes and survival

Number of variant genotypes	<i>n</i>	PFS months	<i>p</i> <sup>a</sup>	Age adjusted HR (95% CI)	BCSS months	<i>p</i> <sup>a</sup>	Age adjusted HR (95% CI)
0 + 1	38	13.3		1.0 Reference	27.5		1.0 Reference
2	29	9.8	0.02 <i>p</i> <sub>trend</sub> = 0.005	1.56 (0.94–2.60)	22.9	0.01 <i>p</i> <sub>trend</sub> = 0.006	1.34 (0.81–2.23)
≥3	28	9.1		<b>2.09 (1.24–3.49)</b>	18.5		<b>2.20 (1.31–3.70)</b>

<sup>a</sup> Log-rank for comparison among Kaplan Meier survival curves. “Risk” genotypes were defined as SOD2-01 TT, MPO-02 GG + AG, GSTP1-01 GG + AG and GSTP1-02 CT + TT. There were two patients with 0 “risk” genotypes and two patients with all 4 “risk” genotypes



**Fig. 1** Kaplan–Meier survival curves of increasing numbers of “risk” genotypes for (A) PFS progression free survival, *p* = 0.02 (B) BCSS breast cancer specific survival, *p* = 0.01. “Risk” genotype groups were defined as: **0,1** [none (*n* = 2): SOD2-01 (CC or CT), GSTP1-01 (AA) GSTP1-02 (CC) and MPO-02 (AA) or any one (*n* = 36) of SOD2-01 (TT), GSTP1-01 (GG or AG), GSTP1-02 (TT or CT) or MPO-02 (GG or AG)] and **2** [any two (*n* = 29) of SOD2-01 (TT), GSTP1-01 (GG or AG), GSTP1-02 (TT or CT) or MPO-02 (GG or AG)] and **≥3** [any 3 (*n* = 26) or 4 (*n* = 2) of SOD2-01 (TT), GSTP1-01 (GG or AG), GSTP1-02 (TT or CT) or MPO-02 (GG or AG)]. *p*-Values are log rank and refer to differences among groups

numbers of GSTP1-01 G and GSTP1-02 T alleles were associated with decreased PFS and BCSS (although not significant). A number of studies have shown an association of polymorphisms in various *GST* genes with treatment

response and/or survival outcomes in breast [33, 39, 40], bladder [41], esophageal [35], gastric [42], lung [43] leukaemia [44] and colorectal [45–47] cancer. The G (Val) allele and/or the GG genotype of GSTP1-01 was associated with increased survival in patients with colorectal [45], ovarian [6] and breast cancer [4, 33] whereas other studies have reported an association of the GSTP1-01 G allele with decreased survival for breast [48] and esophageal cancer [35].

Contradictory findings between various studies may be a result of differences in the patient population including cancer type, stage, chemotherapy regimen and dose. In one study, different genotypes were associated with survival outcome depending on specific treatment groups [34]. In patients with multiple myeloma, the GSTP1-01 AA genotype was associated with improved PFS following treatment with HDC (cyclophosphamide, vincristine, adriamycin and methylprednisolone followed by high-dose melphalan) and ASCT whereas in the standard treatment arm, the G allele (105Val) [adriamycin, BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea), cyclophosphamide and melphalan] was associated with better PFS.

The GSTP1-01 SNP codes for a less efficient substrate binding site in the enzyme and results in decreased detoxification of some chemotherapeutic drugs. In vitro, the GSTP1-01 and GSTP1-02 alleles differ significantly in their ability to protect against the cytotoxicity caused by an alkylating drug such as thiotepa or the platinum drugs, cisplatin and carboplatin. *Escherichia coli* transformed with the GSTP1\*C alleles (GSTP1-01 G and GSTP1-02 T) were significantly more resistant to both cisplatin and carboplatin whereas cells transformed with the GSTP1\*A allele (GSTP1-01 A and GSTP1-02 C) were significantly more resistant to thiotepa [32].

Although a major strength of this study is that all 95 patients had stage IV breast cancer, there are a number of limitations including the small number of patients and incomplete information concerning hormone receptor status for some patients. Information on tissue HER-2 status and post-HDC treatment(s) for these patients was also not available (although there were no planned treatments and any subsequent treatments for progression were palliative).

In conclusion, the results of this study suggests that polymorphisms in genes involved in oxidative stress and drug detoxification may influence survival outcome following chemotherapy treatment with high doses of drugs. We have previously reported that polymorphisms in DNA repair genes may also be associated with survival outcome for this group of patients [36]. Presently, there are few prognostic/predictive factors for patients with MBC and treatment goals are directed towards palliation primarily using a number of DNA damaging agents. Studies examining the influence of genetic variation on survival in various treatment regimens are important in that they may identify patients who can benefit more from a specific treatment. This exploratory study may provide direction for future larger prospective studies with the ultimate goal of improving treatment outcomes for women with MBC. Further analysis of these SNPs in standard treatment settings for both primary and MBC may provide important information towards the development of tailored treatment strategies.

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