

Nucleotide excision repair polymorphisms and survival outcome for patients with metastatic breast cancer

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Abstract

Purpose Inter-individual variations in treatment efficacy may be influenced by polymorphisms in DNA repair genes. We investigated the association of 3 functional polymorphisms in the nucleotide excision repair (NER) pathway with survival outcome of 95 patients with metastatic breast cancer (MBC) treated with DNA-damaging chemotherapy. **Methods** *ERCC1 8092 C/A*, *ERCC2 Asp312Asn* and *ERCC2 Lys751Gln* were determined using Taqman-based genotyping assays. Genotype associations with breast cancer-specific survival (BCSS) and progression-free survival (PFS) were evaluated using Kaplan–Meier estimates and hazard ratios calculated using Cox regression analysis. Tests for trend were conducted by calculating *P*-values for the HR coefficient in proportional hazards regression models.

Results *ERCC2 Lys751Gln* was significantly associated with BCSS (median: 24.8 months for AA/AC combined and 14.2 months for CC, HR: 1.9 (95% CI 1.06–3.26)). Median BCSS decreased with increasing number of designated adverse genotypes for the 3 polymorphisms ($P_{\text{trend}} = 0.003$). Risk estimates for PFS were nonsignificantly elevated and were significantly elevated for BCSS for patients with 2 (HR = 2.21, 95% CI: 1.04–4.72) or 3 (HR = 6.67, 95% CI: 2.19–20.29) adverse genotypes. In treatment subgroup analysis, risk estimates for BCSS were significantly elevated for patients with 3 adverse genotypes

treated with cyclophosphamide, mitoxantrone and vinblastine (HR: 11.9, 95% CI 1.77–79.51) and $P_{\text{trend}} = 0.02$ for increasing number of adverse genotypes. Risk of progression was significantly increased for patients with 1 adverse genotype treated with cyclophosphamide, mitoxantrone and carboplatin (HR: 3.5, 95% CI 1.19–10.6) and $P_{\text{trend}} = 0.02$ for increasing number of adverse genotypes.

Conclusion Polymorphisms in NER pathway may impact survival outcome for patients with MBC following treatment with DNA-damaging chemotherapy. These results provide support for a polygenic pathway approach for assessing the prognostic and predictive potential of polymorphisms in treatment outcome.

Keywords Nucleotide excision repair · Single nucleotide polymorphisms · Metastatic breast cancer · Survival

Introduction

Metastatic breast cancer (MBC) is diagnosed in approximately 5% of new patients, and 30% of previously treated patients will develop recurrent metastatic disease (Gonzalez-Angulo et al. 2007). Presently, few effective treatments for MBC are available, and most patients show either poor or limited responses to treatment. As a result, MBC is considered incurable, and the primary treatment objectives are disease control and palliation. The 5-year survival rate for patients with MBC is approximately 20%, and the median survival duration varies from 12 to 24 months (Gonzalez-Angulo et al. 2007).

There is considerable inter-individual variation in response and survival following chemotherapy and radiation

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treatment of cancer. DNA damage from these treatments can initiate a number of cellular pathways involved in DNA repair, cell cycle control, metabolism and apoptosis. Single nucleotide polymorphisms (SNPs) in genes in these pathways may significantly affect DNA repair efficiency (Au et al. 2004; Abdel-Rahman and El-Zein 2000; Duell et al. 2000), drug toxicity and clinical outcomes following treatment with cancer drugs and/or radiation (Booton et al. 2006; Ambrosone et al. 2006; Gurubhagavatula et al. 2004; Kamikozuru et al. 2008). The influence of individual gene polymorphisms on treatment outcome may be moderate, but reports from our laboratory (Bewick et al. 2006, 2008) and others (Gurubhagavatula et al. 2004; Ambrosone et al. 2005; Nagle et al. 2007; Nowell et al. 2005) suggest that combinations of polymorphisms in several genes may significantly impact clinical responses and survival.

The nucleotide excision repair (NER) pathway is important for DNA repair, removing adducts caused by UV irradiation and environmental agents such as tobacco smoke (polycyclic aromatic hydrocarbons). This pathway is also the main mechanism for the repair of bulky DNA adducts generated by platinum drugs (Altaha et al. 2004) and cyclophosphamide (Andersson et al. 1996) and may be involved in the repair of nonbulky DNA lesions that result from oxidative damage (Hutsell and Sancar 2005). In tumor tissue, low levels of NER gene expression are associated with increased survival for a number of cancers following platinum-based treatments (Azuma et al. 2007; Handra-Luca et al. 2007; Lee et al. 2007; Simon et al. 2007).

Genes involved in the NER pathway code for at least 16 proteins that form a complex nucleotide excision repairosome (Reed 2005). Key genes of the NER pathway include *ERCC1* (excision repair cross-complementation group 1) and *ERCC2* (excision repair cross-complementation group 2, XPD). *ERCC2*, a 5' DNA helicase, is a component of the multi-subunit transcription factor TFIIH that unwinds the DNA duplex in the immediate area of the lesion. The endonuclease, *ERCC1*, is involved in excising the damage to facilitate repair.

Studies suggest that polymorphisms in the NER pathway influence survival outcome in lung (Gurubhagavatula et al. 2004), esophageal (Bradbury et al. 2009), colorectal (Le Morvan et al. 2007) and bone (Caronia et al. 2009) cancer following various DNA-damaging treatments. Functional polymorphisms in these NER genes have been reported that may affect DNA repair capacity and influence the efficacy of treatments using DNA-damaging agents (Wolfe et al. 2007; Au et al. 2003; Lunn et al. 2000). In the *ERCC1* gene, the *ERCC1 8092 C/A* (rs3212986) polymorphism occurs with relatively high frequency. This polymorphism is located in the 3'-untranslated region and may affect mRNA stability (Chen et al. 2000). It is associated with survival outcome for patients with lung and

esophageal cancer treated with cisplatin (Zhou et al. 2004; Wu et al. 2008; Bradbury et al. 2009).

A number of studies have examined two common polymorphisms in the *ERCC2* gene, *ERCC2 Asp312Asn* (rs1799793) and *ERCC2 Lys751Gln* (rs13181). These polymorphisms are associated with variations in adduct levels (Benhamou and Sarasin 2005), chromosomal aberrations (Au et al. 2004; Affatato et al. 2004) and mRNA expression levels in lymphocytes (Wolfe et al. 2007).

Few studies have examined the relationship of these polymorphisms with survival outcome for patients with breast cancer. Therefore, we investigated the association of *ERCC1 8092 C/A* (rs3212986), *ERCC2 Asp312Asn* and *ERCC2 Lys751Gln* with breast cancer-specific survival (BCSS) and progression-free survival (PFS) in 95 patients with metastatic breast cancer (MBC). These patients were enrolled in studies of high-dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT).

Materials and methods

Patient population

The patient population consisted of women that were eligible and had participated in the clinical trials of high-dose chemotherapy and autologous stem cell transplantation. Eligibility criteria for these clinical trials included female patients with histologically proven breast cancer. Age eligibility was restricted to women between 18 and 56 years. Patients had metastatic (stage IV) breast cancer not previously treated with chemotherapy or treated with adjuvant chemotherapy or hormonal therapy and who had progressive disease at least 6 months after completion of adjuvant chemotherapy. Patients were hormone receptor negative or were hormone receptor positive and had progressed on hormone therapy or had rapidly progressing disease where response to hormone treatment could not be awaited.

Patient characteristics, HDC treatment regimens, follow-up and survival information were obtained from clinical research records. These are listed in Table 1 and have been previously described (Bewick et al. 2006, 2008). The clinical trials and study were approved by the Research Ethics Board, Sudbury Regional Hospital, Sudbury, Ontario, and informed signed consent was obtained from all patients.

Analysis of polymorphisms

Apheresis blood product, peripheral blood and bone marrow samples were prospectively collected during treatment, frozen and stored for future use. DNA was isolated from samples using the DNA Blood MiniKit (Qiagen, Mississauga, Ontario) following the manufacturer's protocol. The

Table 1 Clinical characteristics and chemotherapy

Clinical characteristic	Number of patients
Total	95
Median age (range)	45 (19–56)
ER (<i>n</i> = 84)	
Negative	35
Positive	49
PR (<i>n</i> = 80)	
Negative	41
Positive	39
Number of metastatic sites	
UD, 1	54
≥2	41
Metastatic sites ^a	
Bone	53
Lung	32
Lymph node	26
Liver	14
Other	22
HDC regimen	
Cyclo, Mitox, Vin	27
Cyclo, Mitox, Carbo	21
Cyclo, Mitox, Paclitaxel	42
Thiotepa, Cyclo, Carbo	4
Cyclo, Mitox	1

ER estrogen receptor, PR progesterone receptor, UD unassessable disease, HDC high-dose chemotherapy, Cyclo cyclophosphamide (cumulative dose (CD): 6 g/m²), Mitox mitoxantrone (CD: 70 mg/m²), Vin vinblastine (CD: 12 mg/m²), Carbo carboplatin (CD: 800 mg/m²), paclitaxel (CD: 250–400 mg/m²), thiotepa (CD: 500 mg/m²)

^a Number determined at enrollment to study includes metastasis to more than one site

National Cancer Institute SNP500Cancer database (<http://snp500cancer.nci.nih.gov/snplist.cfm>) provided information on polymorphisms (target sequence, frequency estimates and referenced, validated 5'-nuclease assays). Genotypes were determined using TaqMan[®] SNP genotyping assays and the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, Ca.). For quality control purposes, 25% of the samples were randomly selected and reanalyzed at least twice for each polymorphism: the results were 100% concordant. Assignment of genotypes was performed independently by two investigators blinded to the survival endpoints.

Statistical analysis

For each polymorphism, median survival was calculated for each genotype and genotype pairs assessed in codominant, dominant and recessive models. The final model was

selected by combining the genotypes with similar median survivals for each polymorphism. Fisher's exact test was used to evaluate the association between patient and tumor characteristics (age group, ER status, PR status, site and number of metastatic sites) and genotype or genotype combination for each polymorphism. Analyses included two endpoints: breast cancer-specific survival (BCSS) and progression-free survival (PFS). BCSS was defined as 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 abstracted from clinical research records. Progression-free survival (PFS) was defined as time (months) from study registration until documented progression of metastatic disease or censorship (not progressed during follow-up time period). In general, patients were assessed every 2–3 months for the first 2 years and then every 4–6 months thereafter until evidence of progression.

Survival curves were generated using the Kaplan–Meier product limit estimate of the survivorship function. Equality of survivorship function was assessed using the log-rank test. The Cox proportional hazard regression model defined hazard ratios (HR) and 95% confidence intervals (CI). All models were adjusted for age. Genotypes were assigned as “adverse” based on examination of median survival, HR and survival curves of various models of BCSS. Tests for trend were conducted by calculating the *P*-value for the coefficient in a proportional hazards regression models using the adverse variable coded as an ordinal variable. A “risk” score was obtained by summing the number of assigned adverse genotypes for each individual and analyzed for groups of patients with 0, 1, 2 and 3 adverse genotypes. Statistical analysis was done using Stata version 10.1 (Stata Corporation, College Station, TX).

Results

Patient characteristics

The median age at study entry was 45 years (range, 19–56). Disease progression occurred in 91 patients (96%), and 91 patients (96%) died due to MBC, 4 patients remained alive at final follow-up. 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. Genotypes were not associated with ER status, PR status, bone, liver, lung and “other” metastases or number

Table 2 Genotype associations and survival outcome

Polymorphism	<i>N</i>	Median PFS (months)	<i>P</i> **	HR (95% CI)	Median BCSS (months)	<i>P</i> **	HR (95% CI)
ERCC2 Lys751Gln							
AA	45	8.9	0.23	1.0*	19.9	0.08	1.0*
AC	34	10.9		0.7 (0.44–1.11)	26.8		0.8 (0.49–1.22)
CC	16	9.8		1.2 (0.64–2.12)	14.2		1.7 (0.93–3.00)
AA/AC	79	10.4	0.39	1.0*	24.8	0.04	1.0*
CC	16	9.8		1.4 (0.78–2.43)	14.2		1.9 (1.06–3.26)
ERCC2 Asp 312Asn							
GG	44	9.3	0.48	1.0*	18.6	0.22	1.0*
AG	37	10.5		0.8 (0.52–1.29)	26.6		0.8 (0.52–1.29)
AA	14	9.1		1.2 (0.65–2.21)	12.7		1.4 (0.75–2.58)
GG/AG	81	10.4	0.34	1.0*	24.8	0.13	1.0*
AA	14	9.1		1.3 (0.74–2.34)	12.7		1.5 (0.86–2.74)
ERCC1 8092 C/A							
CC	56	9.8	0.07	1.0*	18.5	0.08	1.0*
AC	32	10.8		0.7 (0.42–1.07)	25.1		0.6 (0.38–0.97)
AA	7	8.3		1.7 (0.77–3.85)	26.6		1.2 (0.54–2.69)
AA/AC	39	10.5	0.32	1.0*	26.6	0.09	1.0*
CC	56	9.8		1.3 (0.84–2.00)	18.5		1.5 (0.96–2.26)

* Reference value, *SNP* single nucleotide polymorphism, *P*** log rank, *HR* age-adjusted hazard ratio, *PFS* progression-free survival, *BCSS* breast cancer-specific survival

of metastatic sites with the exception of the *ERCC2 Asp312Asn* GG/AG genotype group that was associated with lymph node metastases compared to patients with the AA genotype ($P = 0.01$).

Patients were assigned to 4 major groups based on differences in HDC treatment regimens (a fifth group contained only 1 patient) (Table 1). There were no significant differences in PFS and BCSS (Kaplan–Meier survival and log-rank analysis of survivorship function) between major treatment groups, patients receiving 1 or 2 ($n = 6$) HDC treatments or patients treated with or without carboplatin.

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

Patients with more than one metastatic site ($n = 41$) had significantly decreased PFS ($P = 0.002$) but not BCSS ($P = 0.15$) compared to the combined patient group with unassessable disease (UD) or 1 metastatic site.

Genotypes, PFS and BCSS

The genotype frequencies and HRs for PFS and BCSS are shown in Table 2. Significant differences for BCSS were observed for the *ERCC2 Lys751Gln* polymorphism. Median survival of patients with the *ERCC2 Lys751Gln* AA/AC combined genotypes was 24.8 months compared to 14.2 months for patients with the *ERCC2 Lys751Gln* CC genotype (HR:1.9, 95% CI 1.06–3.26). *ERCC1 8092 C/A* and *ERCC2 Asp312Asn* polymorphisms were not significantly associated with PFS or BCSS, and the *ERCC2 Lys751Gln* polymorphism was not significantly associated with PFS. *ERCC2 Lys751Gln* CC, *ERCC2 Asp312Asn* AA and *ERCC1 8092C/A* CC were assigned as adverse genotypes based on the analysis of HRs, Kaplan–Meier survival

Table 3 Number of adverse genotypes and BCSS and PFS

# of risk variants	0	1	2	3	<i>P</i>
Median BCSS	26.8 (27)	22.9 (54)	14.1 (10)	7.0 (4)	$\chi^2_{(3)} = 13.8$ $P = 0.003$
Median PFS	10.5 (27)	9.8 (54)	8.8 (10)	5.5 (4)	$\chi^2_{(3)} = 2.8$ $P = 0.43$

Median times are in months, *P* log rank, number in parentheses refers to number of patients, adverse genotypes: *ERCC2 Lys751Gln* CC, *ERCC2 Asp312Asn* AA, *ERCC1 8092 C/A* CC

Table 4 Treatment groups, increasing number of adverse genotypes and BCSS

Number of adverse genotypes	All treatments (<i>n</i> = 95) HR (95% CI)	Cyclo, mitox, paclitaxel (<i>n</i> = 42) HR (95% CI)	Cyclo,mitox, carboplatin (<i>n</i> = 21) HR (95% CI)	Cyclo,mitox, vinblastine (<i>n</i> = 27) HR (95% CI)
0	1.0*	1.0*	1.0*	1.0*
1	1.5 (0.94–2.51)	1.2 (0.61–2.42)	2.7 (0.96–7.32)	1.6 (0.35–7.01)
2	2.2 (1.04–4.72)	1.9 (0.53–6.93)	2.6 (0.29–23.20)	1.8 (0.34–9.84)
3	6.7 (2.19–20.29)	–	–	11.9 (1.77–79.51)
<i>P</i> (trend)	0.002	0.35	0.07	0.02

* Reference value, *HR* age-adjusted hazard ratio, *cyclo* cyclophosphamide, *mitox* mitoxantrone
 Bold values indicate statistical significance

Table 5 Treatment groups, increasing number of adverse genotypes and PFS

Number of adverse genotypes	All treatments (<i>n</i> = 95) HR (95% CI)	Cyclo, mitox, paclitaxel (<i>n</i> = 42) HR (95% CI)	Cyclo,mitox, carboplatin (<i>n</i> = 21) HR (95% CI)	Cyclo,mitox, vinblastine (<i>n</i> = 27) HR (95% CI)
0	1.0*	1.0*	1.0*	1.0*
1	1.4 (0.83–2.25)	0.89 (0.45–1.8)	3.5 (1.19–10.6)	1.2 (0.25–5.24)
2	1.7 (0.78–3.61)	1.1 (0.29–4.03)	4.0 (0.42–37.9)	1.4 (0.24–7.81)
3	2.5 (0.83–7.40)	–	–	3.1 (0.48–19.3)
<i>P</i> (trend)	0.06	0.90	0.02	0.16

* Reference value, *HR* age-adjusted hazard ratio, *cyclo* cyclophosphamide, *mitox* mitoxantrone
 Bold values indicate statistical significance

curves and associations reported in other studies (Le Morvan et al. 2007; Petty et al. 2007; Chew et al. 2009). Table 3 shows the effect on BCSS and PFS with increasing number of adverse genotypes. Median BCSS and PFS progressively decreased for patients with increasing number of adverse genotypes with significant test of trend for BCSS ($P = 0.003$) (Table 3). The risk of death (BCSS) was increased for patients with 1 adverse genotype (HR: 1.53, 95% CI 0.94–2.51) and was significantly increased for patients with 2 adverse genotypes (HR: 2.21, 95% CI 1.04–4.72) and with 3 adverse genotypes (HR: 6.67, 95% CI 2.19–20.29) when compared to patients with no adverse genotypes ($P_{\text{trend}} = 0.002$) (Table 4). For PFS, results were not significant but were suggestive of increased risk of progression with increasing number of adverse genotypes ($P_{\text{trend}} = 0.06$) (Table 5). Fig. 1 shows the Kaplan–Meier survival curves for BCSS for patients grouped according to the number of adverse genotypes.

Subgroup analysis of BCSS and PFS by treatment

Results of studies suggest that polymorphisms in the NER pathway may be important predictors of survival following the treatment of patients with platinum drugs. Therefore, we explored the influence of these polymorphisms in three major HDC treatment groups: patients treated with cyclophosphamide, mitoxantrone and carboplatin (*n* = 21);

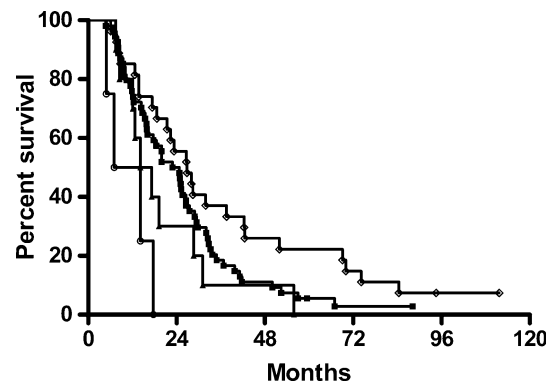


Fig. 1 Kaplan–Meier survival curves for BCSS for patients with 0 (empty diamond), 1 (filled square), 2 (filled triangle) and 3 (empty circle) adverse genotypes. Adverse genotypes were designated as *ERCC2 Lys751Gln CC*, *ERCC2 Asp312Asn AA*, *ERCC1 8092 C/A CC*

patients treated with cyclophosphamide, mitoxantrone and paclitaxel (*n* = 42); and patients treated with cyclophosphamide, mitoxantrone and vinblastine (*n* = 27). For BCSS, the results, shown in Table 4, suggest that for all treatment groups, patients with 1 or more adverse genotype were at increased risk and results were significant for patients with 3 adverse genotypes and treated with cyclophosphamide, mitoxantrone and vinblastine and $P_{\text{trend}} = 0.02$ (Table 4). For PFS, the results, shown in

Table 5, suggest that patients with 1 or more adverse genotypes treated with cyclophosphamide, mitoxantrone and carboplatin ($n = 21$) were at increased risk of progression with a significant test of trend ($P_{\text{trend}} = 0.02$).

Discussion

Polymorphisms in genes involved in DNA repair and drug-related pathways may significantly influence clinical outcome and thus may help identify patients that can benefit from various treatments. This study examined the association of 3 polymorphisms in two genes in the NER pathway with survival outcome for patients with MBC treated with HDC using DNA-damaging drugs. In univariate analysis, results were suggestive of associations of the polymorphisms with increased risk of PFS and BCSS but only the *ERCC2 Lys751Gln* polymorphism was significantly associated with BCSS (HR:1.9, 95% CI1.06–3.26). In combined analyses of all 3 polymorphisms, median PFS and BCSS progressively decreased for patients with increasing number of adverse genotypes and the risk of death (BCSS) was significantly increased for patients with 2 or more adverse genotypes. Further analysis of treatment subgroups suggests that the risk of death was increased for patients in each of the three major treatment subgroups and was significant for the patient group with 3 adverse genotypes that received cyclophosphamide, mitoxantrone and vinblastine.

Overall, these results are biologically plausible and consistent with the role of the NER pathway in the repair of platinum-DNA adducts (Wang and Lippard 2005) and DNA damage resulting from cyclophosphamide (Andersson et al. 1996) and mitoxantrone (Durr et al. 1983). In addition, some studies have reported associations between polymorphisms in NER genes and cancer cell sensitivity in response to taxanes and to vinca alkaloids such as vinblastine (Le Morvan et al. 2006), although these may be mediated indirectly (Zhang et al. 1999; Reed et al. 1995). It is also possible that the association of these polymorphisms with BCSS overall and for treatment subgroups may indicate a potential role as prognostic factors. It is also interesting that these polymorphisms were strongly associated with PFS (Table 5) for the subgroup of patients that received carboplatin. This suggests that these polymorphisms may be predictive of outcome for platinum therapy.

The significant impact on survival outcome with increasing number of adverse genotypes within DNA repair pathways has been reported for other cancers. This includes patients with head and neck cancer following radiation treatment (Carles et al. 2006) and cisplatin treatment (Quintela-Fandino et al. 2006), and also for patients with esophageal cancer (Wu et al. 2006), and NSCLC treated

with platinum-based chemotherapy (Wu et al. 2008; Gurubhagavatula et al. 2004). In vitro, the sensitivity of peripheral blood lymphocytes (measured by the number of chromatid breaks) to the mutagen benzo(α)pyrene diol epoxide (BPDE) increased with increasing number of designated adverse alleles in the NER pathway. These polymorphisms, when analyzed individually, had only moderate effects on mutagen sensitivity (Lin et al. 2007).

We used study-defined criteria to assign potential adverse genotypes as presently there are no definitive assignments of adverse genotypes for these polymorphisms and conflicting associations have been reported in the literature. A similar rationale has been used in previous studies (Lin et al. 2007).

Several studies report associations of *ERCC2 Lys751Gln* AA genotype with decreased DNA repair capacity (Vodicka et al. 2004; Lunn et al. 2000) and therefore it may be associated with a more favorable outcome following DNA-damaging treatment, while others report that the *ERCC2 Lys751Gln* Gln (C allele) variant is associated with decreased repair (Benhamou and Sarasin 2005; Matullo et al. 2001; Spitz et al. 2001; Hou et al. 2002; Rzeszowska-Wolny et al. 2005). In vitro, cancer cells with the *ERCC2 751Gln* CC genotype are more sensitive to taxanes and to vinca alkaloids, such as vinblastine (Le Morvan et al. 2006), suggesting that patients with the CC genotype should have better survival following these drug treatments. However, the results of clinical studies suggest that the *ERCC2 751Gln* CC genotype is predictive of poorer survival for patients with metastatic colorectal cancer treated with the platinum drug, oxaliplatin (Le Morvan et al. 2007), advanced NSCLC patients treated with docetaxel and gemcitabine (Petty et al. 2007) and MBC patients treated with gemcitabine and cisplatin (Chew et al. 2009). Conflicting results are also reported for the *ERCC1 8092 C/A* polymorphism. The A allele was significantly associated with poorer overall survival for patients with advanced NSCLC treated with platinum-based chemotherapy (Zhou et al. 2004) whereas in other studies, the A allele was associated with significantly improved overall survival for patients with esophageal (Bradbury et al. 2009) and NSCLC (Wu et al. 2008) as well as increased gastrointestinal toxicity for patients with NSCLC (Suk et al. 2005) treated with platinum drugs.

These inconsistent results may be due to differences between studies of cancer type and stage, patient population, combinations of drugs and treatments, types of DNA damage biomarkers examined and their method of assessment. Future research to validate potential biomarkers will be challenging since it is becoming more apparent that DNA repair pathways are complex with cross-functionality between pathways that may be differentially regulated and activated in various tissues and by different drugs and drug combinations (Zhang et al. 2009; Herrera et al. 2009).

We have attempted to follow the guidelines of the reporting recommendations for tumor marker prognostic studies (REMARK) (McShane et al. 2005). This is an exploratory retrospective study using a patient population of women who participated in several clinical trials of HDC and autologous stem cell transplantation. Study information was collected from clinical records rather than prospectively recorded. As such, there are a number of limitations of this study. The collection of information was not standardized and as a result is incomplete or missing for some patients for important clinical variables such as hormone receptor status and post-HDC treatments. In addition, the selection and number of polymorphisms examined was not comprehensive. In future prospective studies, it would be important to examine other potentially functional polymorphisms in NER and other DNA repair and cellular pathways appropriate for the drug combinations used. The sample size of this group of patients is small, limiting interpretation of the results, a limitation noted in similar studies. Although it is possible that the results of this study may be due to the high doses of drugs used, it should be noted that survival outcomes for patients treated with HDC are similar to patients treated with standard-dose chemotherapy regimens containing DNA-damaging agents (Farquhar et al. 2007). Also, the results of studies of patients with other cancers indicate that these and other polymorphisms in the NER pathway are important for the efficacy of DNA-damaging agents in standard-dose treatment regimens (Le Morvan et al. 2007; Gurubhagavatula et al. 2004; Bradbury et al. 2009).

In conclusion, these results suggest that combinations of polymorphism in NER may influence survival outcomes in patients with MBC following treatment with DNA-damaging drugs. To our knowledge, only one other study has examined polymorphisms in DNA repair pathways with clinical outcomes for metastatic breast cancer (Chew et al. 2009). Their results also suggest that polymorphisms in DNA repair genes may play an important role in clinical outcomes for patients with MBC following treatment with cisplatin and gemcitabine and similar to this study, the *ERCC2 Lys751Gln* polymorphism was associated with survival outcome. Future prospective studies examining combinations of polymorphisms in DNA repair and drug-related pathways in patients receiving standard-dose treatments are required to determine their usefulness for the development of personalized treatment strategies for MBC.

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Conflict of interest statement We declare that we have no conflict of interest in relation to this work.

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