available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

The role of glutathione-S-transferase polymorphisms in ovarian cancer survival

Christina M. Nagle[a], *Georgia Chenevix-Trench*[b], *Amanda B. Spurdle*[b,*], *Penelope M. Webb*[a]

[a]*Cancer and Population Studies Division, Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Qld 4029, Australia*
[b]*Cancer and Cell Biology Division, Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Qld 4029, Australia*

ARTICLE INFO

Article history:
Received 31 July 2006
Received in revised form 18 September 2006
Accepted 19 September 2006
Available online 3 November 2006

Keywords:
Glututathione-S-transferase
Ovarian cancer
Survival

ABSTRACT

Resistance to chemotherapy represents one of the most important causes of treatment failure in patients with ovarian cancer. Common polymorphisms in the glutathione-S-transferase (*GSTM1, GSTP1 and GSTT1*) family have been implicated in chemoresistence and ovarian cancer survival. In this study, we have analysed Australian women diagnosed with primary invasive epithelial ovarian cancer between 1985 and 1997, using DNA extracted from peripheral blood and archival uninvolved (normal) tissues. *GSTP1* genotypes were determined using ABI Prism 7700 Sequence Detection System methodology ($n = 448$) and *GSTT1* and *GSTM1* genotypes using PCR-agarose methodology ($n = 239$). We observed a significant survival advantage among carriers of *GSTP1 Ile105Val GG/GA* genotype (HR 0.77, 95% confidence interval (CI) 0.61–0.99, $p = 0.04$) and a non-significant survival advantage among women who were homozygous for the *GSTM1* and *GSTT1* deletion variants. There was also evidence of an additive effect, with a stronger survival benefit in women carrying three low function *GST* genotypes (*GSTM1* null, *GSTT1* null and *GSTP1 GA/GG*) (HR 0.47, 95% CI 0.22–1.02). The results of this study, the largest to date, are consistent with a number of previous smaller studies which have also observed that reduced *GST* function was associated with better survival outcomes in patients with ovarian cancer.

1. Introduction

Survival following a diagnosis of ovarian cancer is universally poor. Current treatment includes aggressive surgery, removal of the tumour and resection of metastases with the goal of optimal surgical cytoreduction of tumour to <1 cm before the administration of platinum-based chemotherapy.[1] Whilst the majority of patients achieve a favourable clinical response initially, most develop recurrent cancer and 5-year survival rates are as low as 20%.[2] Despite the fact that residual disease is an important prognostic factor, some patients whose tumours are optimally debulked develop *de novo* resistance to chemotherapy and have poor survival.[3] At the other extreme, a small minority of women whose tumours are suboptimally debulked are sensitive to chemotherapy and never relapse.[4] This range of outcomes is intriguing and may be attributable, at least in part, to sequence variations in genes encoding drug metabolism enzymes, such as common polymorphisms in the glutathione-S-transferase M1, T1 and P1 enzymes (encoded by *GSTM1*, *GSTT1* and *GSTP1*, respectively).[5]

The *GST* enzymes catalyse the conjugation of glutathione with a variety of electrophilic compounds, including cytotoxic

* *Corresponding author:* Tel.: +61 7 3362 0371; fax: +61 7 3362 0105.
E-mail address: Amanda.Spurdle@qimr.edu.au (A.B. Spurdle).
0959-8049/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2006.09.011

agents.[6,7] Three of the GST genes *GSTP1*, *GSTM1* and *GSTT1* have been found to have functional polymorphisms that are frequently present in the general population.[8,9] These polymorphisms either decrease or abolish *GST* enzyme activity. A single nucleotide substitution (A > G) at position 313 of the *GSTP1* gene, which results in replacement of isoleucine with valine at amino acid position 104, substantially diminishes *GSTP1* activity, probably due to decreased enzyme stability.[10–12] Inherited homozygous deletions of the *GSTT1* and *GSTM1* gene lead to the complete absence of enzyme activity.[13–15] There is some evidence that *GST* polymorphisms may play a role in the response to treatment and survival from some cancers[16,17], including cancer of the ovary.[5,18–20] In particular, *GSTP1* has been shown to interact with platinum-based compounds[21] and glutathione-conjugated platinum can be quickly effluxed from cells.[22] Thus, it has been suggested that high *GST* activity may result in more rapid drug metabolism that diminishes the cytotoxic effects of chemotherapy on tumour cells, and hence be associated with poor treatment response and worse survival.[18]

GSTP1 expression has been shown to be associated with less favourable response and survival in some studies[19,20,23] although no such relationship has been detected in other studies.[24,25] Many of these studies have however been small, and adjustment for clinical factors has varied.

Given the biochemical evidence that the *GST* enzymes are involved in the detoxification of platinum based cytotoxic agents, we hypothesised that decreased activity of the *GST* detoxification pathway (due to null or reduced expression of particular *GST* enzymes) could make some ovarian cancers more sensitive to chemotherapy and hence be associated with better survival. Therefore, we analysed common polymorphisms in the *GSTP1*, *GSTM1*, and *GSTT1* in a large group of women diagnosed with ovarian cancer between 1985 and 1997 to determine whether the presence of one or more of these polymorphisms was associated with survival.

2. Patients and methods

2.1. Subjects

This study included 454 women, aged 18–80 years diagnosed with primary incident invasive epithelial ovarian cancer between 1985 and 1997. Just over two thirds of the women (n = 296 [67%]) had participated in an Australian population based case-control study, the Survey of Women's Health (SWH) between 1990 and 1993. The methods have been described previously.[26] Briefly, these women were ascertained through major gynaecology–oncology treatment centres in the three most populous Australian states: Queensland, New South Wales and Victoria. A central gynaecologic histopathologist reviewed all the pathology reports and sections of each tumour to confirm the diagnosis and histological subtype. The remaining women (n = 158) were ascertained as incident cases from the Royal Brisbane Hospital, Queensland, Australia, between 1985 and 1997, as described previously.[27,28]

Information on diagnosis, disease stage (using the International Federation of Gynaecologists and Obstetricians (FIGO) criteria), tumour histology, grade and treatment was abstracted retrospectively from the women's medical records and pathology reports or, for a subset of cases, from the Royal Brisbane Hospital Gynaecology Oncology database. Ethics approval for the research was received from the Queensland Institute of Medical Research, Royal Brisbane Hospital and the metropolitan hospitals where the women were originally diagnosed and treated.

The SWH cohort was followed for mortality using personal identifiers which were linked to state cancer registry records and the Australian National Death Index (NDI). Both the NDI and cancer registries used probabilistic record linkage software to match the women to their databases. For incident cases from the Royal Brisbane Hospital, information on survival status was obtained from the hospital Gynaecology Oncology database, and the Queensland Cancer registry.

2.2. Genotype analysis

DNA preparation and genotyping (including repeatability and other quality control measures) was as described previously.[27,28] Briefly, germline DNA was extracted from peripheral blood by a salt-precipitation method for women recruited through the Royal Brisbane Hospital, and from uninvolved tissue archival paraffin blocks for women ascertained through the population-based study. ABI Prism 7700 Sequence Detection System (SDS) methodology was used for genotyping the *GSTP1* A to G Ile105Val variant (rs1695) and PCR-agarose methodology was used to detect the *GSTT1* and *GSTM1* deletion variants. The assay design and methodology used for detection of the deletion variants precluded the reliable genotyping of poorer quality DNA samples extracted from archival blocks, thus *GSTT1* and *GSTM1* genotypes were obtained only from peripheral blood DNA (n = 239). As described previously, there was no deviation from Hardy–Weinberg equilibrium for the GSTP1 genotype, and allele frequencies for all three loci were similar to those reported previously for Caucasian populations.[28]

2.3. Statistical analysis

Survival time was calculated from the date of diagnosis to the date of death (from ovarian cancer) or censored at 1st September 2004 or death from another cause. The Kaplan–Meier technique was used to plot crude survival curves and estimate crude overall survival probabilities, and adjusted hazard ratios (HRs) and 95% confidence intervals (95% confidence intervals (CIs)) were obtained from Cox regression models. The p-value for linear trend was calculated by the change in likelihood ratio statistic for entry of a linear term in the model, and thus was a χ^2-test on 1 degree of freedom. All analyses were adjusted for age (10-year age groups), tumour stage, histologic subgroup, and histologic grade. Tumour grades 2 (moderate), 3 (poor) and 4 (undifferentiated) were combined into a single group because survival did not vary for these three sub-groups, but was significantly different from that for women with grade 1 (well differentiated) tumours. For the small number of women with missing information on grade (n = 24), grade was imputed (for the purpose of adjustment only) based on the histologic subtype of their tumour according to the distribution among women with known histologic subtype and grade. All analyses were

performed using the Statistical Packages for Social Sciences for Windows, version 13.0 (SPSS Inc., Chicago, IL).

3. Results

Among the 454 women with invasive epithelial ovarian cancer, 288 (63%) had died from the disease during the follow-up period. The crude 5-year survival for these women was 44% (standard error (SE) = 2%). As expected a number of the clinical and pathological factors were clearly associated with survival in the crude analyses, but in a multifactorial model the only factors that remained significant were older age at diagnosis, late FIGO stage, histological subgroup and higher histologic grade (Table 1). These four factors were included in all subsequent models. Further adjustment for other variables shown to influence survival in crude analyses (e.g. amount of residual disease and platinum based chemotherapy) made no difference to the estimates of effect.

The results of analyses focusing on the detoxification genes (*GSTM1*, *GSTT1*, *GSTP1*) are shown in Table 2 and Fig. 1. Most notable were the inverse associations with the *GSTT1* and *GSTM1* null genotypes (Fig. 1a and b) and possession of a *GSTP1 G* allele (Fig. 1c). The absolute survival advantages were 18% for the *GSTM1* null genotype, 18% for the *GSTT1* null genotype and 23% for possession of one or more *G* alleles for *GSTP1*. We repeated these survival analyses in a smaller, but more homogeneous group of women, those with the most common histologic subtype and FIGO stages (serous and FIGO stages III and IV disease), and found the same associations (results not shown). Additional analysis restricted to patients who had received platinum based chemotherapy (75% of the sample), adjusted for age, FIGO stage, histologic subgroup and grade, also found evidence of a similar survival advantage for patients with *GSTM1* null genotype (HR 0.73, 95% CI 0.52–1.03), *GSTT1* null genotype (HR 0.84, 95% CI 0.55–1.27), one or more G alleles for *GSTP1* (HR 0.84, 95% CI 0.65–1.10). The individual sample sizes for women who received alternative chemotherapy, or no chemotherapy, were too small to investigate possible differences in genetic associations between these treatment types.

The combined effects of the aforementioned *GST* polymorphisms on survival are shown in Table 3 and Fig. 2. Of note

Table 1 – Association between clinical and pathological factors and ovarian cancer survival

	n[a]	n dead	Crude 5-year survival (%)	Crude HR (95% CI)	Adjusted[b] HR (95% CI)
Age group					
<50 yrs	106	52	57		
50–59 yrs	123	78	49	1.37 (0.97–1.95)	1.09 (0.74–1.62)
60–69 yrs	131	90	38	1.84 (1.31–2.59)	1.34 (0.91–1.97)
70+	94	68	29	2.30 (1.60–3.30)	1.57 (1.04–2.36)
					p trend 0.16
FIGO stage					
I	83	12	86		
II	44	14	76	2.43 (1.12–5.26)	1.92 (0.82–4.47)
III	278	217	31	10.32 (5.76–18.51)	6.99 (3.49–13.98)
IV	41	83	11	19.17 (9.96–36.90)	11.79 (5.37–25.86)
					p trend <0.01
Histological subgroup					
Serous	274	207	35		
Mucinous	29	8	78	0.25 (0.12–0.51)	1.02 (0.52–2.34)
Endometrioid	62	22	67	0.32 (0.20–0.49)	0.56 (0.33–0.87)
Clear cell	31	14	57	0.51 (0.29–0.87)	1.88 (1.04–3.40)
Other	58	37	42	0.77 (0.54–1.10)	0.78 (0.52–1.15)
					p trend 0.01
Grade					
1	56	17	74		
2	128	73	49	2.54 (1.49–4.31)	1.80 (1.01–3.19)
3–4	241	181	35	3.86 (2.34–6.37)	1.96 (1.12–3.44)
					p trend 0.08
Residual disease					
< 1 cm	241	139	51		
1–2 cm	47	36	27	1.79 (1.24–2.60)	1.07 (0.72–1.60)
>2 cm	49	43	20	2.31 (1.63–3.26)	1.41 (0.95–2.09)
					p trend 0.03
Platinum based chemotherapy					
Yes	340	242	38		
No	63	25	64	0.42 (0.27–0.63)	0.58 (0.37–0.92)

a Column numbers (*n*) do not sum to total because some data are missing.
b Adjusted for age group, FIGO stage, grade, platinum based chemotherapy.

Table 2 – Association between detoxification genes and ovarian cancer survival

	n	*n* dead	Crude 5-year survival (%)	Crude HR (95% CI)	Adjusted[a] HR (95% CI)
GSTM1[b]					
Non-null	108	82	28	1.0	1.0
Null	131	93	38	0.77 (0.57–1.04)	0.82 (0.59–1.14) $p = 0.25$
GSTT1[c]					
Non-null	190	142	31	1.0	1.0
Null	49	33	44	0.75 (0.52–1.11)	0.82 (0.54–1.23) $p = 0.34$
GSTP1[d]					
AA	182	125	39	1.0	1.0
GA	211	125	47	0.77 (0.60–0.99)	0.77 (0.59–1.00)
GG	55	32	51	0.72 (0.49–1.07)	0.79 (0.53–1.17) $p = 0.13$
GG/GA	266	157	48	0.76 (0.60–0.97)	0.77 (0.61–0.99) $p = 0.04$

a Adjusted for age group, FIGO stage, histologic subtype, histologic grade (1 *versus* 2–4).
b *GSTM1* analyses $n = 239$.
c *GSTT1* analyses $n = 239$.
d *GSTP1* analyses $n = 448$.

was the better survival in women with all three low function *GST* genotypes (*GSTM1* null, *GSTT1* null and *GSTP1 AG/GG*). This group had a 53% survival advantage compared to women with *GSTM1* non-null, *GSTT1* non-null and *GSTP1 AG/GG* genotypes (HR 0.47, 95% CI 0.22–1.02). There was also a suggestion of a modest, but statistically non-significant (p 0.19), inverse trend toward a gene-dose effect illustrated by a greater protective effect for those with three low function polymorphisms, followed by those with two low function polymorphisms (HR 0.67, 95% CI 0.40–1.13) and 1 allele (0.82, 95% CI 0.51–1.32). Again, there was little material difference in HRs when the analysis was limited to the subgroup of women who had platinum based therapy (HR for *GSTM1* null, *GSTT1* null and *GSTP1 AG/GG* genotype = 0.52, 95% CI 0.26–1.02). Analysis restricted to patients with serous histology and FIGO stages III and IV disease showed similar results (HR for *GSTM1* null, *GSTT1* null and *GSTP1 AG/GG* genotype = 0.45, 95% CI 0.19–1.10).

4. Discussion

This study suggests that genetic variability in *GST* is independently associated with survival in a large cohort of patients with invasive epithelial ovarian cancer who mostly received platinum based chemotherapy. Patients who carried the null deletions in the *GSTT1* and *GSTM1* genes had moderate survival benefits compared with patients with high expression, and lower function *GSTP1* genotypes also conferred a survival advantage. We also found an inverse, but statistically non-significant, association in patients carrying a combination of all three low function polymorphisms (*GSTM1* null , *GSTT1* null and *GSTP1 AG/GG*). Additional analyses restricted to women treated with platinum based chemotherapy made little material difference to these results, but these patients did dominate the sample set.

The women in this analysis were unselected with regard to genotype and follow-up was essentially complete. The cause of death and baseline clinical measures were made independently of laboratory analyses, and are likely to have been accurate. Laboratory analyses were unbiased with respect to outcome and, although some non-differential misclassification (random error) will be inevitable, the likely effect of this would be to bias our results towards the null, thus the true effects might be greater than those seen here. Similarly ascertainment of outcome is thought to be almost complete and did not vary by genotype or other clinical factors. A potential limitation of the study is the possibility of selection bias introduced by cases who did not participate in the original case-control study due to illness or death ($n = 69$), but this is unlikely to have been an issue for incident cases recruited through the Royal Brisbane Hospital, since recruitment was at the time of clinical diagnosis with no additional requirements for participation such as collection of detailed questionnaire information. It is also likely that women who did not participate in the original case-control study would have been more ill and had worse survival than those who did take part, thus the overall survival proportions observed here might over-estimate survival among all women with ovarian cancer. This would not, however, have affected our results unless the association between *GST* genotype and survival somehow differed between participants and non-participants. We have adjusted for the major clinical factors that affect survival but there is likely to be some residual confounding because of the imperfect nature of some of the clinical measures. However, it is unlikely that this would be sufficient to completely explain the observed associations. The 95% CI and p-values suggest that the effects of null deletions of *GSTT1* and *GSTM1* could be due to chance. To overcome this we have presented the additive effects model which we expect will be replicated by other larger studies.

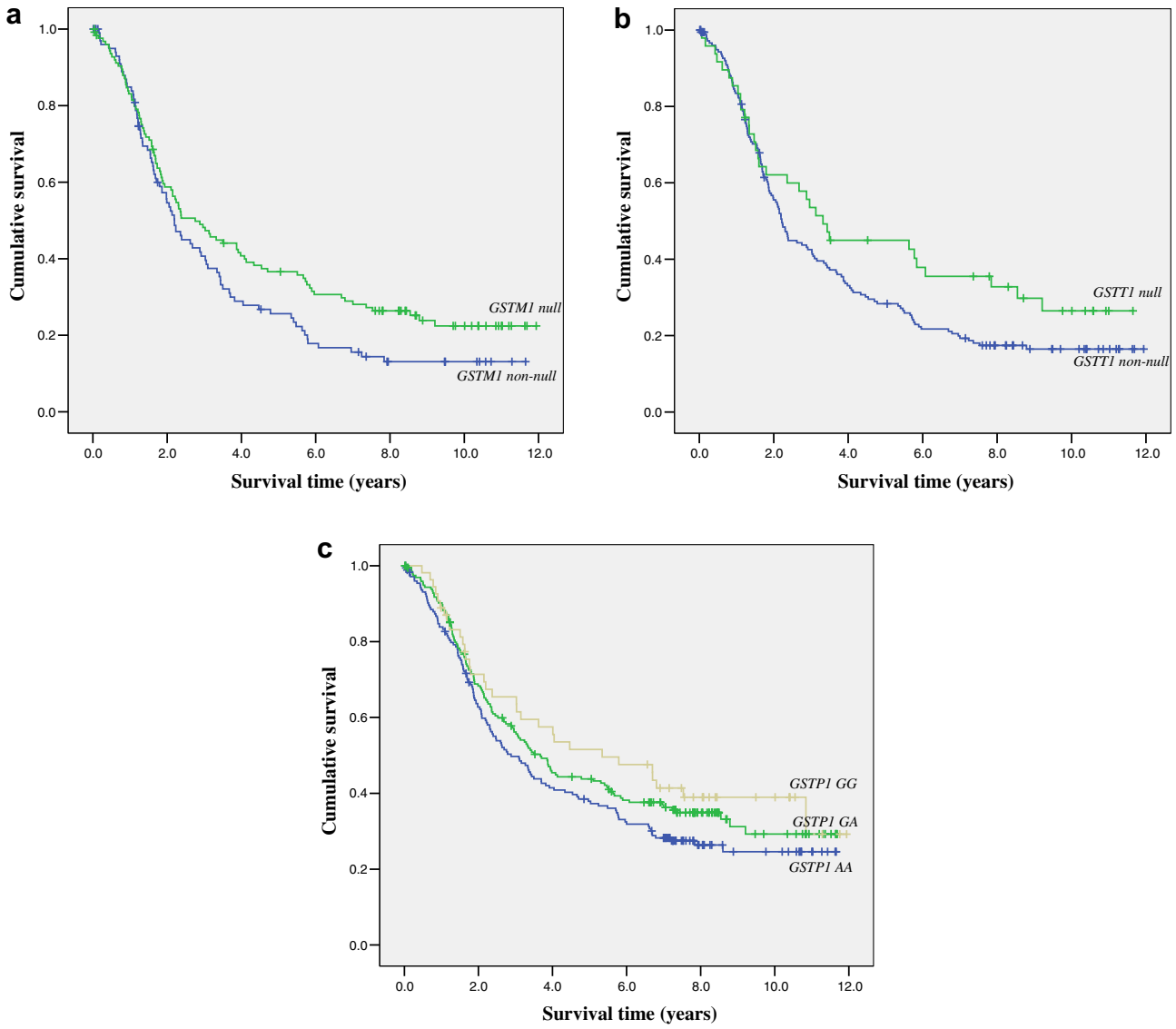

Fig. 1 – (a) Kaplan–Meier survival curves for *GSTM1* genotypes; *GSTM1* null, *GSTM1* non-null. (b) Kaplan–Meier survival curves for *GSTT1* genotypes; *GSTT1* null, *GSTT1* non-null. (c) Kaplan–Meier survival curves for *GSTP1* genotypes; *GSTP1 GG*, *GSTP1 GA*, *GSTP1 AA*.

Table 3 – Association between the number of low function *GST* variants (*GSTT1* null, *GSTM1* null, *GSTP1 AG/GG*) and ovarian cancer survival

Number of low-function variants	*n*	*n* dead	Crude 5-year survival%	Crude HR (95% CI)	Adjusted[a] HR (95% CI)
0[b]	32	24	14		
1[c]	111	89	28	0.90 (0.57–1.92)	0.82 (0.51–1.32)
2[d]	81	51	45	0.56 (0.34–0.92)	0.67 (0.40–1.13)
3[e]	15	11	38	0.61 (0.30–1.26)	0.47 (0.22–1.02)
					p trend = 0.19

a Adjusted for age group, FIGO stage, histologic subtype, histologic grade (1 *versus* 2–4).
b 0, *GSTM1* non-null and *GSTT1* non-null and *GSTP1 AA*.
c 1, *GSTM1* null or *GSTT1* null or *GSTP1 GA/GG*.
d 2, *GSTM1* and *GSTT1* null or *GSTM1* null and *GSTP1 GA/GG* or *GSTT1* null and *GSTP1 GA/GG*.
e 3, *GSTM1* null and *GSTT1* null and *GSTP1 GA/GG*.

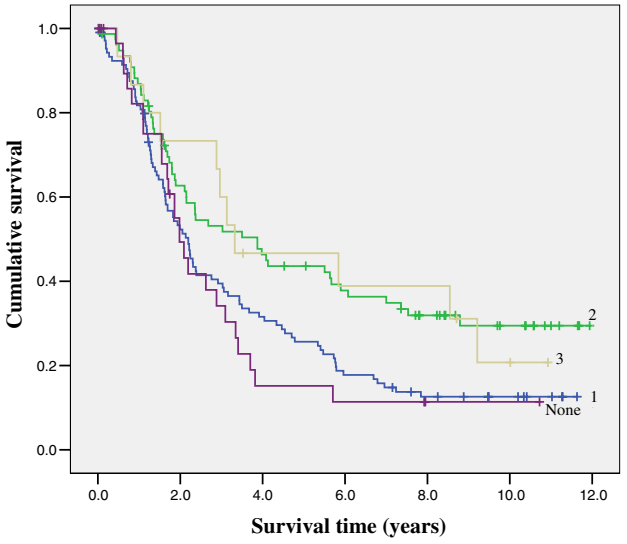

Fig. 2 – Kaplan–Meier survival curves for the number of low function *GST* variants (*GSTT1* null, *GSTM1* null, *GSTP1 AG/GG*). None, *GSTM1* non-null and *GSTT1* non-null and *GSTP1 AA*; 1, *GSTM1* null or *GSTT1* null or *GSTP1 GA/GG*; 2, *GSTM1* and *GSTT1* null or *GSTM1* null and *GSTP1 GA/GG* or *GSTT1* null and *GSTP1 GA/GG*; 3, *GSTM1* null and *GSTT1* null and *GSTP1 GA/GG*.

There have been a number of studies of *GST* genetic polymorphisms and outcomes in ovarian cancer. The most recent and largest study to date by Beeghly and colleagues[18] examined the association between polymorphisms in *GSTM1*, *GSTT1* and *GSTP1*, disease progression and survival in women with primary, invasive epithelial ovarian cancer. Among 215 women with ovarian cancer they found that after adjustment for age, FIGO stage and grade, *GSTM1* null patients were less likely to die compared to patients with *GSTM1* (HR 0.68, 95% CI 0.45–1.03). Furthermore, they reported that women with no *GSTM1* and a lower function *GSTP1* genotype (Ile/val or val/val) had better progression-free survival (HR 0.42, 95% CI 0.24–0.75) and overall survival (HR 0.61, 95% CI 0.36–1.05). Similar findings were seen in patients with *GSTM1* null, *GSTT1* null and *GSTP1* low function compared to present or full function (HR 0.47, 95% CI 0.17–1.28). A number of other studies have also reported beneficial effects of GST genotypes of no or low activity on cancer survival, but for the most part these studies have been small and carried out among heterogeneous populations.[5,19,20,23] Medeiros and colleagues[5] reported better disease free interval and survival for the *GSTM1* null genotype; however this was a very small study of only 24 women with primary ovarian cancer. An association between elevated IHC-detected expression of *GSTP1* and a less favourable response to treatment with cisplatin has been previously reported in groups of 213 cases[19] and 117 cases,[20] and Surowiak and colleagues[23] recently found that shorter survival time was linked to cases of higher expression GST-pi at first look laprotomy.

Some studies however have reported no relationship or opposite associations between *GST* polymorphisms and survival. In a study of 146 women with invasive epithelial ovarian cancer, Lallas and colleagues[29] reported no difference in survival of women with advanced stage epithelial ovarian cancer genotyped as *GSTMI* null compared to those who were *GSTM1* positive. A number of other studies (with sample sizes less than 100) have also failed to find any association between *GSTP1* expression and outcome in ovarian cancer.[24,25,30] Howells and colleagues[31] genotyped 148 women with epithelial ovarian cancer and found no association between the *GSTTI* or *GSTM1* null genotypes and survival individually, but did find poorer outcome in women with a combination of *GSTM1* and *GSTT1* null genotypes (HR 3.44, 95% CI 1.67–7.09). This study included patients with non-invasive disease and the results were not adjusted for potential confounders. In another small study ($n = 81$) of *GSTP1* and ovarian cancer survival, Howell and colleagues[32] reported a significant association between the GSTP1 Ile^{104}/Ile^{104} (AA) and Ile^{104}/Val^{104} (AG) genotypes and improved survival. However, these findings are inconsistent with protein expression findings reported in the same study, in that better survival was associated with negative or decreased GSTP1 protein expression, which functional studies would predict to be associated with the *GSTP1 G* variant encoding a less stable protein.[33]

In this study, we found that subjects with the null or low *GSTP1* activity had better survival, which is consistent with the findings of Beeghly and colleagues[18] and others[5,19,20,23]. Our study has been the largest to date, and the comprehensive follow-up of our sample set minimises the chance of bias. Given that the association we observed was modest, we would suggest that the failure to report similar findings in many of the smaller studies may simply be a reflection of the poor power to detect a relatively modest effect. It is possible that this association is mechanistically related to decreased metabolism of chemotherapeutic agents in general; however we had no power to assess the effect of these variants in other treatment groups, and given that platinum-based chemotherapy is the norm, extremely large studies would be required to test this specific hypothesis. Interestingly, Beeghly and colleagues[18] did report that the survival benefits seen in their cohort became more evident when they stratified by specific chemotherapeutic agents. Of the *GST* polymorphisms examined, *GSTM1* null patients survived best across all chemotherapy subgroups; platinum HR 0.63 (95% CI 0.41–0.96), taxol and platinum HR 0.35 (95% CI 0.15–0.84) and cyclophosphamide and platinum HR 0.51 (95% CI 0.25–1.02). They also found that the *GSTP1* genotype had the strongest effect on patients treated with platinum and cyclophosphamide, known substrates of the GSTP1 enzyme (HR 0.50, 95% CI 0.25–1.03).[18]

Our analysis of ovarian cancer patients, well characterised with respect to survival status and other relevant clinical information, provides evidence for a potential role of *GST* polymorphisms in survival. It implies that the clinical course of patients may in part be genetically determined due to altered GST function. Our findings are consistent with our hypothesis that women with decreased *GST* detoxification would have better survival due to improved response to chemotherapy. We suggest that large well-designed studies would be required to attempt to replicate our evidence for additive effects of genetic polymorphisms in the *GST* pathway, given the increased power required for such interactions. We also suggest that improved genotyping

methodology that can discriminate between heterozygote and homozygote deletion carriers may better estimate risks association with the *GSTT1* and *GSTM1* deletion variants. If replicated, these results certainly warrant more critical testing by further clinical studies and *in vivo* investigations concerning the mechanism of this polymorphic effect, and ultimately allow for genotyping to be performed in the clinical setting to individualise and optimise ovarian cancer treatment.

Conflict of interest statement

None declared.

Acknowledgements

We are grateful to the physicians, surgeons and oncologists who endorsed this project, and the many women who participated in this research. We thank Xiaoqing Chen for assistance with genotyping, and Lee Tripcony for assistance with data retrieval for this project.

Sources of support: This study was supported in part by a grant from the Mayne Bequest Fund, University of Queensland. Initial funding to recruit the cases for the Survey of Women's Health was obtained from the National Health & Medical Research Council (NHMRC) and Queensland Cancer Fund, and funding for genotyping was from the NHMRC. P. Webb is funded by a Queensland Cancer Fund Senior Research Fellowship, A. Spurdle is funded by an NHMRC Career Development Award and G. Chenevix-Trench is a NHMRC Principle Research Fellow.

REFERENCES

1. Guppy AE, Nathan PD, Rustin GJ. Epithelial ovarian cancer: a review of current management. *Clin Oncol* 2005;**17**:399–411.
2. Heintz AP, Odicino F, Maisonneuve P, et al. Carcinoma of the ovary. *Int J Gynaecol Obstet* 2003;**83**(Suppl. 1):135–66.
3. Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. *J Clin Oncol* 2002;**20**:1248–59.
4. Berchuck A, Iversen ES, Lancaster JM, et al. Patterns of gene expression that characterize long-term survival in advanced stage serous ovarian cancers. *Clin Cancer Res* 2005;**11**:3686–96.
5. Medeiros R, Pereira D, Afonso N, et al. Platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma: glutathione *S*-transferase genetic polymorphisms as predictive biomarkers of disease outcome. *Int J Clin Oncol* 2003;**8**:156–61.
6. Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-*S*-transferase family of enzymes. *Mutat Res* 2001;**482**:21–6.
7. Ban N, Takahashi Y, Takayama T, et al. Transfection of glutathione *S*-transferase (GST)-pi antisense complementary DNA increases the sensitivity of a colon cancer cell line to adriamycin, cisplatin, melphalan, and etoposide. *Cancer Res* 1996;**56**:3577–82.
8. Garte S, Gaspari L, Alexandrie AK, et al. Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:1239–48.
9. Rebbeck TR. Molecular epidemiology of the human glutathione *S*-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. *Cancer Epidemiol Biomarkers Prev* 1997;**6**:733–43.
10. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione *S*-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. *J Biol Chem* 1997;**272**:10004–12.
11. Harries LW, Stubbins MJ, Forman D, Howard GC, Wolf CR. Identification of genetic polymorphisms at the glutathione *S*-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis* 1997;**18**:641–4.
12. Zimniak P, Nanduri B, Pikula S, et al. Naturally occurring human glutathione *S*-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. *Eur J Biochem* 1994;**224**:893–9.
13. Board PG. Biochemical genetics of glutathione-*S*-transferase in man. *Am J Hum Genet* 1981;**33**:36–43.
14. Pemble S, Schroeder KR, Spencer SR, et al. Human glutathione *S*-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J* 1994;**300**:271–6.
15. Seidegard J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on *trans*-stilbene oxide are due to a gene deletion. *Proc Natl Acad Sci USA* 1988;**85**:7293–7.
16. Stoehlmacher J, Park DJ, Zhang W, et al. Association between glutathione *S*-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. *J Natl Cancer Inst* 2002;**94**:936–42.
17. Yang G, Shu XO, Ruan ZX, et al. Genetic polymorphisms in glutathione-*S*-transferase genes (GSTM1, GSTT1, GSTP1) and survival after chemotherapy for invasive breast carcinoma. *Cancer* 2005;**103**:52–8.
18. Beeghly A, Katsaros D, Chen H, et al. Glutathione *S*-transferase polymorphisms and ovarian cancer treatment and survival. *Gynecol Oncol* 2006;**100**:330–7.
19. Mayr D, Pannekamp U, Baretton GB, et al. Immunohistochemical analysis of drug resistance-associated proteins in ovarian carcinomas. *Pathol Res Pract* 2000;**196**:469–75.
20. Satoh T, Nishida M, Tsunoda H, Kubo T. Expression of glutathione *S*-transferase pi (GST-pi) in human malignant ovarian tumours. *Eur J Obstet Gynecol Reprod Biol* 2001;**96**:202–8.
21. Goto S, Iida T, Cho S, Oka M, Kohno S, Kondo T. Overexpression of glutathione *S*-transferase pi enhances the adduct formation of cisplatin with glutathione in human cancer cells. *Free Radic Res* 1999;**31**:549–58.
22. Harpole DH, Moore MB, Herndon JE, et al. The prognostic value of molecular marker analysis in patients treated with trimodality therapy for esophageal cancer. *Clin Cancer Res* 2001;**7**:562–9.
23. Surowiak P, Materna V, Kaplenko I, et al. Augmented expression of metallothionein and glutathione *S*-transferase pi as unfavourable prognostic factors in cisplatin-treated ovarian cancer patients. *Virchows Archiv* 2005;**447**:626–33.
24. van der Zee AG, Hollema H, Suurmeijer AJ, et al. Value of P-glycoprotein, glutathione *S*-transferase pi, c-erbB-2, and p53 as prognostic factors in ovarian carcinomas. *J Clin Oncology* 1995;**13**:70–8.
25. Ikeda K, Sakai K, Yamamoto R, et al. Multivariate analysis for prognostic significance of histologic subtype, GST-pi, MDR-1,

and p53 in stages II–IV ovarian cancer. *Int J Gynecol Cancer* 2003;**13**:776–84.
26. Purdie D, Green A, Bain C, et al. Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. Survey of Women's Health Study Group. *Int J Cancer* 1995;**62**:678–84.
27. Spurdle AB, Purdie DM, Webb PM, Chen X, Green A, Chenevix-Trench G. The microsomal epoxide hydrolase Tyr113His polymorphism: association with risk of ovarian cancer. *Mol Carcinog* 2001;**30**:71–8.
28. Spurdle AB, Webb PM, Purdie DM, Chen X, Green A, Chenevix-Trench G. Polymorphisms at the glutathione S-transferase GSTM1, GSTT1 and GSTP1 loci: risk of ovarian cancer by histological subtype. *Carcinogenesis* 2001;**22**:67–72.
29. Lallas TA, McClain SK, Shahin MS, Buller RE. The glutathione S-transferase M1 genotype in ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2000;**9**:587–90.
30. Wrigley EC, McGown AT, Buckley H, Hall A, Crowther D. Glutathione-S-transferase activity and isoenzyme levels measured by two methods in ovarian cancer, and their value as markers of disease outcome. *Br J Cancer* 1996;**73**:763–9.
31. Howells RE, Redman CW, Dhar KK, et al. Association of glutathione S-transferase GSTM1 and GSTT1 null genotypes with clinical outcome in epithelial ovarian cancer. *Clin Cancer Res* 1998;**4**:2439–45.
32. Howells RE, Dhar KK, Hoban PR, et al. Association between glutathione-S-transferase GSTP1 genotypes, GSTP1 over-expression, and outcome in epithelial ovarian cancer. *Int J Gynecol Cancer* 2004;**14**:242–50.
33. Johansson AS, Stenberg G, Widersten M, Mannervik B. Structure–activity relationships and thermal stability of human glutathione transferase P1-1 governed by the H-site residue 105. *J Mol Biol* 1998;**78**:687–98.