Association of **GSTP1** Polymorphism and Survival for Esophageal Cancer

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Abstract

**Purpose:** Activity of glutathione S-transferase (GST) is associated with detoxification of xenobiotics and the maintenance of cell viability. Genetically variant GSTs produce different enzymatic activities. The clinical significance of this variation is still puzzling. We investigated whether genetic polymorphisms of GST including **GSTP1**, **GSTM1**, and **GSTT1** affect survival among esophageal cancer patients.

**Experimental Design:** From 1996 to 2002, 233 patients with pathologically proven esophageal cancer were recruited from the Department of Surgery, National Taiwan University Hospital. GST genotypes, including **GSTT1**, **GSTM1**, and **GSTP1**, were determined by PCR or PCR-RFLP. The influence of the genetic polymorphisms on patient survival was estimated using the method of Kaplan-Meier survival function and Cox proportional hazards models.

**Results:** The mean survival times (months) of the **GSTP1** Ile/Ile, Ile/Val, and Val/Val were 11, 10, and 7, respectively (P < 0.05). The more the patients carried **GSTP1** variant Val alleles, the poorer the survival rate (adjusted hazard ratio, 1.36; 95% confidence interval, 1.01-1.84; P trend = 0.045). In contrast, no association of **GSTT1** or **GSTM1** genotypes with survival rate was noted.

**Conclusion:** The presence of the **GSTP1** variant allele (Val) is associated with a poorer prognosis of esophageal cancer.

Esophageal cancer remains a major public health concern, characterized by a poor prognosis with a 5-year survival rate below 10% (1–6). Squamous cell carcinoma is the predominant cellular type of esophageal cancer worldwide, although the incidence of esophageal adenocarcinoma has been increasing since 1970 in several Western countries (7–10). In seeking to improve its dismal clinical outlook, biochemical targets that are associated with esophageal cancer represent a therapeutic potential. Glutathione S-transferase (GST) activity constitutes an important cellular protection mechanism through reduction of oxidative stress by mediating glutathione transference. GST activity is exhibited by a family of enzymes (α, μ, π, σ, and θ). Enzyme activity can be detected in both normal and cancerous tissues of the esophagus and is not affected by cigarette smoking or alcohol consumption (11). In a report of one patient, complete tumor regression achieved by treatment with 5-fluorouracil plus cisplatin was found associated with the reduced tumor expression of GST-π (12). On the other hand, high expression of GST-π has been linked to poorer survival in esophageal cancer patients receiving chemoradiation followed by surgical resection (13).

In the general population, two variant alleles are found in the **GSTP1** gene, involving a Val-to-Ile amino acid replacement at residue 105. These genetic variants profoundly influence **GSTP1** substrate-specific activity and affinity (14–16). **GST1** and **GSTM1** can even be completely absent, with the resulting abrogation of the enzymatic activities. Genetic variation in GST activity and affinity influences the treatment outcome in patients with cancers of the head and neck, lung, colorectum, breast, and blood (17–20). Previously, we and others have found that polymorphisms of GSTs can influence an individual's susceptibility to develop esophageal cancer (21–23). However, as yet, the clinical outcome of these genetic polymorphisms of GSTs in esophageal cancer is unknown. The present study examined this issue.

**Patients and Methods**

Patients of esophageal cancer in the Department of Surgery of National Taiwan University Hospital in Taiwan from 1996 to 2002 were enrolled in the study. Information regarding the demographics, disease characteristics (tumor location and tumor-node-metastasis, TNM, stage), course of treatment, and vital and recurrence status was obtained by reports obtained from the Tumor Registry of the National Taiwan University and/or pathologic review. After informed consent was obtained, 10 mL of blood were obtained from each patient before treatment. Buffy coat was isolated from the blood and stored in a −70°C freezer for further evaluation. The study has been approved by the ethical committee of the National Taiwan University Hospital.

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For the patients undergoing esophagectomy, tumors located at or above the middle-third thoracic esophagus were resected through a right thoracotomy, with reconstruction via a retrosternal conduit and left cervical esophageal anastomosis (three-field surgery). Tumors at the lower-third thoracic esophagus or near the gastroesophageal junction were resected through a left thoracoabdominal incision and reconstructed via intrathoracic esophagostomy or esophagojejunostomy.

**Laboratory investigation.** Genomic DNA was extracted from peripheral leukocytes with serial processing with RNase and proteinase K followed by ethanol precipitation. For GSTP1 genotyping, PCR-RFLP was used (21). The primers were 5'-ACCCCAAGGGCTCTATGGGAA and 5'-TCAGGCGACAAAGGCCCCT. The PCR product was subsequently digested by the restriction enzyme BsmI (New England Biolabs, Beverly, MA) at 55°C, which was then, together with a DNA size marker (5X174RF DNA/HaeIII fragments), analyzed by electrophoresis on a 3% agarose gel (FMC, Rockland, ME) and visualized by ethidiumbromide staining. The products were classified as Ile/Ile (the predominant homozygote), Ile/Val (heterozygote), and Val/Val (the rare homozygote). GSTM1 polymorphism was analyzed by PCR as previously described (23). Primers of 5'-GAATCTCCCTGAAAGCTAACGC-3' and 5'-GTGGGCTCAAATATACGGTGG-3' were used in the PCR amplification. The primers of β-globulin were used as an internal control. The GSTM1 present genotype produced a 215-bp product that was not visible for the null genotype. Similarly, GSTT1 genotype was also analyzed with PCR (23). Primers of 5'-TCAGGCGATCATGGGAA and 5'-CGGGGCTCAAATATACGGTGG-3' were used. A 480-bp product was acquired for GSTT1 present genotype but not for the null genotype.

**Statistical analysis.** Demographic and clinical characteristics were compared across genotypes, using Pearson χ² or Fisher's exact statistics. Effect of genetic polymorphisms on the survival was estimated using the method of Kaplan-Meier survival and assessed using the log-rank test. Cox proportional hazards models were used to evaluate the effect genetic polymorphisms on the survival after adjusting for other covariates. All statistical analyzed used SAS software version 8 (Cary, NC). Two-sided Ps < 0.05 were considered statistically significant.

### Results

The characteristics of the study population (n = 233) are shown in Table 1. The median age at diagnosis was 62 years (range, 37-92 years). Two hundred and three patients had a diagnosis of squamous cell carcinoma, 20 had adenocarcinoma, and 10 had other cellular types. Among these patients, 165 underwent esophagectomy for the cancer, 140 had three-field dissection (combined thoracotomy, laparotomy, and cervical esophagogastrectomy), and 25 had left thoracoabdominal incision. Of these patients undergoing surgery, 42 (25%) patients had anastomotic leakage, 37 (22.4%) had pulmonary complications, and 17 (10.3%) had other complications. Twelve patients had mortality within 1 month in the hospital (7.2%). One hundred and forty patients received chemotherapy alone or in combination with either radiotherapy or esophagectomy. Cisplatin with 5-fluorouracil or paclitaxol were used as the main chemotherapeutic agents. For neoadjuvant chemotherapy, a dose of 6,000 to 7,000 cGy was used for definite irradiation or chemoradiation.

The distribution of GST genotypes was 71.2% Ile/Ile (n = 166), 25.3% Ile/Val (n = 59), and 3.5% Val/Val (n = 8) for the GSTP1 polymorphism; 56.7% null (n = 132) for the GSTM1 genotype; and 48.9% null (n = 114) for the GSTT1 genotype. The demographic distribution of the genotypes is summarized in Table 1. A significant difference of the GSTP1 genotype distribution was noted according to the modes of surgical intervention (P = 0.02). The distribution of GST genotype in different TNM staging was not evident.

The mean survival times (months) of the patients harboring GSTP1Ile/Ile, Ile/Val, and Val/Val were 11, 10, and 7, respectively (P < 0.05; Fig 1; Table 2). Because the genotype distribution of GSTP1 differed according to the surgical approaches, the mode of surgical resection for esophagectomy was included in the multivariate analysis in the Cox proportional hazards model. After adjusting for age, sex, status of operation, and TNM stages and using the Ile/Ile genotype as reference, the hazard ratios (HR; 95% confidence interval) of Ile/Val and Val/Val was 1.39 (0.96-2.01) and 1.73 (0.67-4.47), respectively (Table 3). Compared with patients with the GSTP1 Ile/Ile genotype, there was a trend of worsening survival with the presence of GSTP1 Val alleles (HR, 1.36; 95% confidence

### Table 1. Clinical profiles and GSTP1 genotype of the esophageal cancer patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (%)</th>
<th>Ile/Ile (%)</th>
<th>Ile/Val (%)</th>
<th>Val/Val (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;50</td>
<td>41</td>
<td>27 (65.8)</td>
<td>11 (26.8)</td>
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<td>50-65</td>
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<td>19 (18.6)</td>
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</tr>
<tr>
<td>&gt;65</td>
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<td>59 (65.6)</td>
<td>29 (32.2)</td>
<td>2 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
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<td>152 (70.7)</td>
<td>56 (26.0)</td>
<td>7 (3.3)</td>
<td>0.46</td>
</tr>
<tr>
<td>Female</td>
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<td>3 (16.7)</td>
<td>1 (5.5)</td>
<td></td>
</tr>
<tr>
<td>T staging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>52</td>
<td>35 (67.3)</td>
<td>16 (30.8)</td>
<td>1 (1.9)</td>
<td>0.28</td>
</tr>
<tr>
<td>II</td>
<td>47</td>
<td>34 (72.3)</td>
<td>10 (21.3)</td>
<td>3 (6.4)</td>
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</tr>
<tr>
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<td>73</td>
<td>53 (72.6)</td>
<td>20 (27.4)</td>
<td>0 (0)</td>
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</tr>
<tr>
<td>IV</td>
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<td>2 (4.7)</td>
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<tr>
<td>N staging</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>95</td>
<td>65 (68.4)</td>
<td>28 (29.5)</td>
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<td>1</td>
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<td>25 (21.9)</td>
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<tr>
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<td>1 (2.5)</td>
<td>0.28</td>
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<td>56 (70.9)</td>
<td>20 (25.3)</td>
<td>3 (3.8)</td>
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<td>I</td>
<td>90</td>
<td>63 (70)</td>
<td>25 (27.8)</td>
<td>2 (2.2)</td>
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<tr>
<td>Nil</td>
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<td>51 (75)</td>
<td>12 (17.6)</td>
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<td>Three-field</td>
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<td>100 (71.5)</td>
<td>39 (27.8)</td>
<td>1 (0.7)</td>
<td></td>
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<tr>
<td>Left</td>
<td>25</td>
<td>15 (60.0)</td>
<td>8 (32)</td>
<td>2 (8)</td>
<td></td>
</tr>
<tr>
<td>thoracoabdominal incision</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Neoadjuvant therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>93</td>
<td>64 (68.8)</td>
<td>24 (25.8)</td>
<td>5 (5.4)</td>
<td>0.43</td>
</tr>
<tr>
<td>Yes</td>
<td>140</td>
<td>102 (72.9)</td>
<td>35 (25)</td>
<td>3 (2.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher's exact test.
†18 missing.
‡20 missing.
§TNM staging according to the proposal of Japanese Committee for Registration of Esophageal Carcinoma (43).
interval, 1.01-1.84), adjusted for age, sex, status of operation, and TNM stages (P_trend = 0.045; Table 3).

Only the TNM stages and GST genotypes seemed to be independent factors affecting the prognosis of esophageal cancer. The Kaplan-Meier function for survival in patients with the GSTP1 genotype is shown in Fig. 1. Only eight patients with the GSTP1 Val/Val genotype were available for analysis and had no survival difference compared with that of other genotypes. However, the survival of patients with GSTP1 Ile/Val genotype was worse than those patients harboring the GSTP1 Ile/Ile genotype. The GSTP1 genotype distribution was not associated with the response to chemoirradiation (data not shown). The effect of GSTT1 and GSTM1 on survival was not evident. The mean survival times of GSTM1 null and present genotypes were 14.5 ± 13.1 and 15.8 ± 16.2 months, respectively. In addition, the mean survival times of GSTT1 null and present genotypes were 16.9 ± 16.1 and 13.2 ± 13.3 months, respectively. The HRs of death for genotypes of GSTM1 and GSTT1 (present versus absent) were 0.93 (P_trend = 0.68) and 1.20 (P_trend = 0.69), respectively.

Discussion

The present results show that genetic polymorphism of GSTP1 may play a significant role in influencing the survival for patients with esophageal cancers. The significantly increased HR of death with the allelic number of GSTP1 Val in the patients of esophageal cancer supports a gene-dose effect of the GSTP1 Val allele on patient survival. However, the survival curve of the patients with GSTP1 Val/Val genotype was variable, perhaps influenced by the very small number of patients in this subgroup.

This study included patients of various treatment modalities. The genotype distribution of GSTP1 showed a significant difference in the different subgroups about surgical treatment. To exclude the confounding influence of the treatment factors, we adjusted the status of surgical approaches and neoadjuvant therapy in multivariate analysis. Under stepwise multivariate analysis, only the presence of GSTP1 Val allele and the TNM staging were independent prognostic significant factors. Other clinical or pathologic factors including tumor grade and treatment modality did not significantly influence the prognosis. In the surgical patients (n = 165), the survival was also not

Table 2. Survival among patients of esophageal cancer categorized with GSTP1 genotype

<table>
<thead>
<tr>
<th>GST genotype</th>
<th>No. patients</th>
<th>Deaths</th>
<th>Crude HRs (95% confidence interval)</th>
<th>Adjusted HRs* (95% confidence interval)</th>
<th>Trend test †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile/Ile</td>
<td>166</td>
<td>98</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Ile/Val</td>
<td>59</td>
<td>42</td>
<td>1.34 (0.93-1.92)</td>
<td>1.39 (0.96-2.01)</td>
<td>1.36 (1.01-1.84)</td>
</tr>
<tr>
<td>Val/Val</td>
<td>8</td>
<td>5</td>
<td>1.45 (0.59-3.57)</td>
<td>1.73 (0.67-4.47)</td>
<td></td>
</tr>
</tbody>
</table>

*HRs from Cox proportional hazards model, after adjusting for age, sex, status of operation, TNM stages, and neoadjuvant therapy.
†Trend test from Ile/Ile, Ile/Val, to Val/Val, P = 0.045.
statistically different between the groups with and without neoadjuvant therapy ($n = 97$ versus $68$; HR, $1.40$; without versus with neoadjuvant therapy; $P = 0.11$) in the univariate analysis.

A previous report of esophageal cancer patients receiving chemoradiation followed by surgical resection showed that expression of GST-π is inversely associated with patients’ survival (13). GST is responsible for the first step in the elimination of many environmental toxins. Through glutathione S-transferase GSTP1 is also responsible for the first step in the elimination of many environmental toxins. Through glutathione S-transferase GSTP1, patients harboring the GSTP1 genotypes remained unchanged after adjusting with other possible survival confounders. Patients harboring the GSTP1 $^{105}$Val allele can have a better clinical outcome for cancers of the breast, colon, or multiple myeloma after receiving chemotherapy (19, 28, 29). On the other hand, a reduced survival in ovarian cancer correlates with the GSTP1 $^{105}$Val/Val genotype, with the best outcome in the GSTP1 $^{105}$Ile/Val (30). The favorable genotype had reduced GST expression; however, it did not associate with response to chemotherapy or tumor staging (30). These collective observations support the idea that the functional alteration by GSTP1 genetic polymorphism might not only influence the resistance to chemotheraphy or irradiation but also other cancer biological behaviors in determining the treatment outcome. In vitro, underexpression of GSTP1 leads to spontaneous or cigarette extract–induced apoptosis in lung fibroblasts (31, 32). GSTP1 can also inhibit c-jun-NH2-kinase activity thus modulating the mitogen-activated protein kinase pathway and therefore influencing cellular proliferation (33, 34). In bladder cancer, the pattern of p53 mutation is associated with GST genetic deletion, although not significantly (35). These observations require further clinical studies before their ultimate clinical significance becomes clear.

Presently, GSTT1 and GSTM1 genetic polymorphisms did not show a significant effect on the survival of esophageal cancer. Previously, patients of breast cancer or Hodgkin lymphoma with null genotypes for GSTM1 and/or GSTT1 were found to have better treatment outcome compared with those with present alleles (36, 37). However, the GSTM1 null genotype compromises the survival in the patients of lung cancer or juvenile leukemia (38, 39). In our patients, the survival in the null and present genotype of GSTM1 and GSTT1 was almost the same. This absence of association might be due to the low expression of GSTT1 and GSTM1 in the gastrointestinal tract (40).

Our study population was limited by the heterogeneity in terms of the treatment modalities and tumor pathology. We evaluated the results of patients with varied cellular type, because previous clinical studies have shown a similar clinical outcome and tumor behavior in the different cellular types of esophageal cancer (41, 42). We could also find a similar trend of survival for GSTP1 polymorphism in the patients of esophageal squamous cell carcinoma. However, the difference was not evident with this number of patients ($n = 203$), with an HR (95% confidence interval) of 1.29 (0.94–1.77; $P_{\text{trend}} = 0.12$ for the GSTP1 variant allele). Some of our patients have palliative chemotherapy and/or irradiation, or best supportive care only. We therefore did not evaluate the disease-free survival or tumor recurrence/metastasis status in this study. Study of the GSTP1 polymorphism’s prognostic effect on the patients with specific cellular type and treatment modality is needed in the future.

In conclusion, our study implicates the GST $^{105}$Ile/Val genetic polymorphism as a significant factor influencing survival in esophageal cancer patients. This implies that the clinical course for the patients of esophageal cancer might be genetically determined due to the altered GST-π function, creating the possibility of improving survival for esophageal cancer by manipulation of its function. However, our preliminary result will require confirmation by more clinical studies and in vitro investigations concerning the mechanism of the polymorphism effect.

References

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