Effect of Genetic Polymorphisms Related to DNA Repair and the Xenobiotic Pathway on the Prognosis and Survival of Gastric Cancer Patients

TOMOMITSU TAHARA[1], TOMOYUKI SHIBATA[1], MASAKATSU NAKAMURA[1], HIROMI YAMASHITA[1], DAISUKE YOSHIOKA[1], MASAAKI OKUBO[1], JOH YONEMURA[1], TAKAMITSU ISHIZUKA[1], NAOKO MARUYAMA[1], TOSHIAKI KAMANO[1], YOSHIO KAMIYA[1], HIROSHI FUJITA[1], YOSHIHITO NAKAGAWA[1], MITSUO NAGASAKA[1], MASAMI IWATA[1], HIDETO YAMADA[2], ICHIRO HIRATA[1] and TOMIYASU ARISAWA[2]

[1]*Department of Gastroenterology, Fujita Health University School of Medicine, Toyoake, Japan;*
[2]*Department of Gastroenterology, Kanazawa Medical University, Ishikawa, Japan*

Abstract. *Background: A number of association studies have focused on the effect of polymorphisms related to DNA repair or the xenobiotic pathway, on the susceptibility to gastric cancer (GC). Here, the possible association between common polymorphisms in the X-ray repair cross-complementing groups (XRCC) 1, and glutathione-S-transferase (GST) genes and various clinicopathological characteristics, including overall survival, in GC patients were evaluated. Patients and Methods: XRCC1 Arg399Gln, and Arg194Trp, GSTP1 Ile104Val, and GSTT1, GSTM1 null polymorphisms were determined in 130 GC patients. Results: XRCC1 codon 194 Trp carriers (Trp/Trp + Arg/Trp) held a significantly higher risk of venous invasion (OR=3.76, 95%CI=1.05-13.51, p=0.043). A similar trend was also found for the XRCC1 codon 194 Trp/Trp genotype (OR=2.15, 95% CI=0.87-5.34, p=0.099). The frequencies of the XRCC1 codon 399 Gln/Gln and Arg/Gln genotypes tended to be lower in lymphatic invasion-positive GC (XRCC1 codon 399 Gln/Gln: OR=0.27, 95% CI=0.06-1.15, p=0.075, Gln/Gln + Arg/Gln: OR=0.46, 95% CI=0.20-1.06, p=0.069), while the frequencies of the XRCC1 codon 194 Trp/Trp genotype tended to be higher in lymphatic invasion-positive GC (XRCC1 codon 194 Trp/Trp: OR=7.70, 95% CI=0.95-62.60, p=0.056). The patients with the GSTT1 null genotype showed significantly better overall survival than the patients with the GSTT1 present genotype (p=0.019). Conclusion: XRCC1 codon 194 Trp carrier status is correlated with more aggressive biological behavior of GC, such as venous invasion, and the GSTT1 null genotype is associated with better survival in GC patients.*

Correspondence to: Tomomitsu Tahara, Current address: 7300 Brompton ST #5134, Houston, TX, 77025, U.S.A. Tel: +731 9276147, e-mail: tomomiccyu@yahoo.co.jp

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Gastric cancer (GC) is one of the most common and lethal malignancies in Japanese and East Asian populations and the second most common cause of cancer-related deaths in the world (1, 2). Although the incidence and mortality rate of GC located outside the cardia have been decreasing over the last few decades, a considerable percentage of patients still have advanced disease at diagnosis and some of them are not indicated for curative surgery.

Although, several mechanisms may be related to susceptibility to GC development there is accumulating evidence that DNA repair or the xenobiotic pathway might be involved in these processes. X-Ray repair cross-complementing groups (XRCCs) are important proteins of the DNA repair pathways. The *XRCC1* gene is responsible for a scaffolding protein that directly associates with other proteins such as DNA polymerase β, PARP (ADP-ribose polymerase) and DNA ligase III in a complex, to facilitate the processes of base excision repair (BER) or single-strand break repair (3). The BER pathway repairs DNA damage caused by a variety of endogenous and exogenous factors, including oxidation, alkylating agents and ionizing radiation (4, 5). The XRCC1 protein can bind directly to both gapped and nicked DNA, as well as to gapped DNA associated with DNA polymerase β, suggesting that this protein might be independently involved in DNA damage recognition (6). Two polymorphisms, more often found in the XRCC1 conserved sites, lead to a C to T substitution at codon 194 in exon 6 and to a G to A substitution at codon 399 in exon 10 of the gene, leading to the amino acid alterations arginine

(Arg) to tryptophan (Trp) and arginine (Arg) to glutamine (Gln), respectively. These changes in conserved protein sites may alter the BER capacity, increasing the chances of DNA damage (7). Both the Arg399Gln, and Arg194Trp variants have been associated with cancer susceptibility (8-12), including GC (13-16).

Glutathione-*S*-transferases (GSTs) are important enzymes of the xenobiotic pathway. These enzymes catalyze the conjugation of potentially mutagenic electrophilic compounds, with nucleophilic glutathione yielding less toxic and more water-soluble compounds, which are readily excreted *via* urine or bile (17). Thus, GSTs protect the body from the harmful effects of carcinogens and a reduction of their activity can render an individual more susceptible to various carcinomas (18). Both *GSTT1* and *GSTM1* genes of the GST super gene family exhibit null or deletion polymorphism (18, 19). Individuals homozygous for the null allele lack GST enzyme activity and hence have an increased risk of cancer (18). *GSTP1* also exhibits a polymorphism within its coding region, leading to an A to G substitution at codon 104 of the gene, leading to the amino acid alteration isoleucine (Ile) to valine (Val), which reduces enzyme activity (20). *GSTM1, GSTT1* and *GSTP1* polymorphisms have also been studied in several malignancies including GC (21-24).

Because of their important roles in DNA repair and the xenobiotic pathway, a number of studies have evaluated the association between *XRCC1* and GST polymorphisms with susceptibility to GC (13-16, 23, 24). In addition, several studies have also investigated the association between these polymorphisms and survival in GC patients, using anticancer drugs (25, 26).

Since *XRCC1* and GST polymorphisms might have important roles in cancer development, we speculated that these biological polymorphisms may also be associated with distinct behavior of GC. In this study, the association between *XRCC1* Arg399Gln and Arg194Trp, *GSTP1* Ile104Val, and *GSTT1* and *GSTM1* null polymorphisms and various clinicopathological characteristics of GC and overall survival were evaluated in a Japanese population.

Patients and Methods

Patients, DNA extraction, and Helicobacter pylori infection status. The studied population comprised 130 patients with GC being treated in our hospitals. All GC cases were diagnosed histologically and were classified according to Lauren's classification. Detailed information was obtained concerning anatomic location, lymph node and distant metastasis, and peritoneal dissemination. Information on venous, and lymphatic invasion was also obtained in 100 out of 107 resected cases. Based on this information, early GC was defined as localized within the mucosa or submucosa, irrespective of lymph node metastasis and all others were defined as advanced GC.

Table I. *Clinicopathological characteristics of GC patients.*

Variable (n)	
Mean age±SD (years)	65.1±12.2
Gender (Male:Female)	89:41
Lauren's histological subtype	
Intestinal type	74
Diffuse type	56
Helicobacter pylori infection status	
H. pylori (+)	101
H. pylori (–)	29
Stage	
Early cancer	58
Advanced cancer	72
Anatomical location	
Upper third	11
Middle third	64
Lower third	55

Among 128 patients, including 23 unresectable and 105 resectable cases, overall survival, defined as the time from the date of surgery for resectable cases and the date of initial chemotherapy for unresectable cases, was recorded. *H. pylori* infection status was assessed by serologic or histological analysis, or urea breath test. Patients were diagnosed as infected when at least one of the diagnostic tests was positive. The Ethics Committee of the Fujita Health University School of Medicine approved the protocol, and written informed consent was obtained from all the participating subjects.

Genotyping for polymorphisms. Genomic DNA was extracted from uninvolved mucosa of the gastric antrum or peripheral blood using the standard phenol/chloroform method in all the patients.

Genotyping for the *XRCC1* gene codons 399 and 194, and *GSTP1* polymorphisms were carried out by multiplex PCR-RFLP, as previously described (9, 27), while the *GSTT1* and *GSTM1* null polymorphisms were detected by PCR, using exon 7 of the constitutional *CYP1A1* gene as internal control, as previously described with slight modifications (28). Instead of multiplex PCR, genotyping for *GSTT1* and *GSTM1* was conducted separately.

Statistical analyses. Genotype frequencies were calculated by direct counting. Logistic regression analysis with adjustment for sex and age was used to assess the association between the clinicopathological characteristics and the genotypes. Survival among different genotypes was assessed using the Kaplan–Meier method and compared using the log-rank test. A probability value of less than 0.05 was considered as statistically significant.

Results

Characteristics of subjects, association between XRCC1 codons 399 and 194, GSTP1, GSTT1, and GSTM1 polymorphisms and clinicopathological characteristics of GC. The characteristics of the 130 GC patients are shown in Table I, and the association between *XRCC1* codons 399 and 194, *GSTP1*, *GSTT1* and *GSTM1* polymorphisms and the

Table II. *Associations between XRCC1, GSTP1, GSTT1 and GSTM1 polymorphisms and various clinicopathological subtypes of GS.*

Variables (n)	*XRCC1* codon 399 genotype			*XRCC1* codon 194 genotype			*GSTP1* genotype			*GSTT1* genotype		*GSTM1* genotype	
	Arg/Arg	Arg/Gln	Gln/Gln	Arg/Arg	Arg/Trp	Trp/Trp	IIe/IIe	IIe/Val	Val/Val	Present	Null	Present	Null
Staging (130 cases)													
Early (58 cases)	30	21	6	30	23	4	37	18	2	18	40	26	32
Advanced (72)	35	30	6	32	31	8	47	24	1	29	43	37	35
Lymphatic invasion (100 cases)[#]													
Negative (39)	16	16	6	19	18	1	27	10	1	14	25	20	19
Positive (61)	36	22	3	27	23	11	39	20	2	16	45	27	34
Venous invasion (130 cases)[$]													
Negative (66)	33	26	7	34	27	5	42	22	2	22	45	30	37
Positive (64)	19	12	2	12	14	7	24	8	1	8	25	17	16
Lymph node metastasis (130 cases)													
Negative (66)	36	22	8	33	26	7	40	23	2	20	46	31	35
Positive (64)	29	29	4	29	28	5	44	19	1	17	37	34	32
Peritoneal dissemination (130 cases)													
Negative (102)	53	40	8	48	42	11	67	31	3	34	68	48	54
Positive (28)	12	11	4	14	12	1	17	11	0	13	15	15	13

Note: *XRCC1* codon 399 and 194 polymorphisms could not be genotyped for two patients. *GSTP1* polymorphism could not be genotyped for one patient. All data were adjusted for age and gender. [#]*XRCC1* codon 399 Gln/Gln: OR=0.27, 95% CI=0.06-1.15, p=0.075, Gln/Gln+Arg/Gln: OR=0.46, 95% CI=0.20-1.06, p=0.069. *XRCC1* codon 194 Trp/Trp: OR=7.70, 95% CI=0.95-62.60, p=0.056. [$]*XRCC1* codon 194 Trp/Trp: OR=2.15, 95% CI=0.87-5.34, p=0.099, Trp/Trp+Arg/Trp: OR=3.76, 95% CI=1.05-13.51, p=0.043.

clinicopathological characteristics of GC are shown in Table II. The *GSTT1*, and *GSTM1* polymorphisms were successfully genotyped for all the patients. The *XRCC1* codon 399 and 194 polymorphisms could not be genotyped for two patients. The *GSTP1* polymorphism could not be genotyped for one patient. The frequencies of the *XRCC1* codon 399 Gln/Gln and Arg/Gln genotypes tended to be lower in lymphatic invasion-positive GC (*XRCC1* codon 399 Gln/Gln: OR=0.27, 95% CI=0.06-1.15, p=0.075, Gln/Gln + Arg/Gln: OR=0.46, 95% CI=0.20-1.06, p=0.069). On the other hand, the frequency of the *XRCC1* codon 194 Trp/Trp genotype tended to be higher in lymphatic invasion-positive GC (*XRCC1* codon 194 Trp/Trp: OR=7.70, 95% CI=0.95-62.60, p=0.056). *XRCC1* codon 194 Trp carriers (Trp/Trp + Arg/Trp) held a significantly higher risk of venous invasion (OR=3.76, 95% CI=1.05-13.51, p=0.043). A similar trend was also found for the *XRCC1* codon 194 Trp/Trp genotype (OR=2.15, 95% CI=0.87-5.34, p=0.099). As shown in Table II, no significant association was found between the *XRCC1* codon 399 and 194, *GSTP1*, *GSTT1* and *GSTM1* polymorphisms and staging, lymph node metastasis and peritoneal dissemination. Moreover, no association was found between the *XRCC1* codon 399 and 194, *GSTP1*, *GSTT1*, and *GSTM1* polymorphisms polymorphisms and the other clinicopathological subtypes, such as Lauren's histological classification, anatomical location, liver and other distant metastasis (data not shown).

Association between survival curves estimated by the Kaplan-Meier method, and XRCC1 codon 399 and 194, GSTP1, GSTT1, and GSTM1 polymorphisms. The median follow-up period of the 128 assessed patients was 30.0 months. Among the 23 unresectable patients, TS-1-based chemotherapy was administered in 15 cases, taxane-based chemotherapy in five cases, and chemotherapy with other regimens in remaining three patients. The patients with the *GSTT1* null genotype showed significantly better overall survival than the patients with *GSTT1* present genotype (p=0.019: Figure 1d), while no association was found between the other genotypes (*XRCC1* codons 399 and 194, *GSTP1* and *GSTM1*) and overall survival (Figure 1 a-c, e).

Discussion

XRCC1 codon 194 Trp carriers (Trp/Trp + Arg/Trp) held a significantly higher risk of venous invasion and a similar trend was also found for the *XRCC1* codon 194 Trp/Trp genotype with both venous and lymphatic invasion. These associations indicated that different GC subtypes may have different genetic backgrounds and the *XRCC1* codon 194 genotype may lead to distinct biological behavior such as venous invasion-positive phenotypes. The *XRCC1* codon 194 Trp allele may thus be associated with more aggressive pathological phenotypes in GC. On the other hand, no association was found between the other polymorphisms and the GC characteristics, except for a

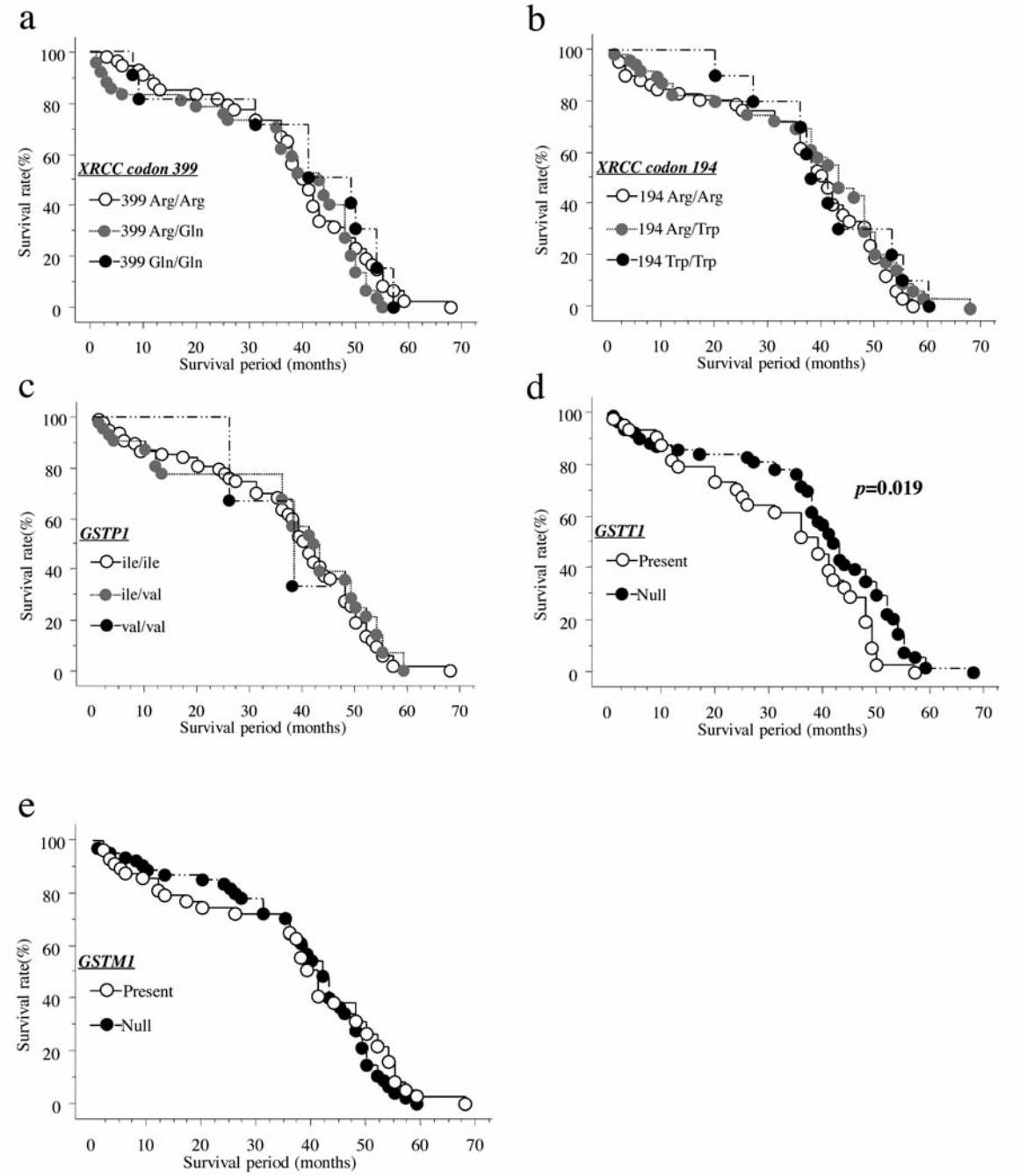

Figure 1. *Association between survival curves estimated by Kaplan-Meier method, with different XRCC1 codons 399 and 194, GSTP1, GSTT1 and GSTM1 genotypes (n=128). Statistical analysis was performed by the log-rank test.*

weak association between the *XRCC1* codon 399 Gln/Gln and Arg/Gln genotypes with reduced risk of lymphatic invasion-positive GC, suggesting a weak effect of these polymorphisms on biological behavior in GC. Inconsistent results have been observed regarding the *XRCC1* 194 Arg and 399 Gln alleles in relation to GC. Lee *et al*. (14) reported that the haplotype combining *XRCC1* 194 Trp, 280 Arg and 399 Arg was associated with a significant reduction in GC risk in Korea, while Huang *et al*. (16) showed an association between the greatest smoking-associated GC risk and the 399 Arg/Arg genotype, but not the 194 genotypes in Poland. The 194 Arg/Arg genotype was associated with a significantly increased risk for cardiac GC in China (13) , while another study in North Central China showed that the *XRCC1* 399 Gln allele

was associated with reduced risk of cardiac GC and the 194 genotype alone was not associated with cardiac GC (15). More recently, Capellá *et al*. (29) showed that the *XRCC1* 399 Gln allele was associated with increased risk of severe gastric atrophy, but association was not observed for the 194 genotypes. These discrepancies may be due to population stratification and different characteristics of the patients, or to the *XRCC1* polymorphism being linked to the real disease-causing variant(s). Together with the current result, it is clear the *XRCC1* genotype may have differing effects on GC susceptibility and progression in different populations, and anatomical locations (cardia and non cardia), reflecting the diversity of the environmental or etiological factors.

The patients with the *GSTT1* null genotype showed significantly better overall survival than the patients with the *GSTT1* present genotype. The deletion genotype in *GSTT1* has also been shown to be associated with poorer prognosis in GC (25). However, in this study, the low enzymatic activity, *GSTT1* null-genotype was associated with rather superior overall survival. Three speculative explanations for the association between the null genotype and improved survival are possible. First, GSTT1 may have a role in metabolizing certain substances which have a protective effect against cancer, and the null genotype may increase the concentration of these substances and, thus, may be associated with superior overall survival. Second, this polymorphism may be in linkage disequilibrium with other polymorphisms elsewhere in the *GSTT1* gene, which influence the overall survival in GC patients, demonstrating biologically relevant variability. Finally, this polymorphism may be in linkage disequilibrium with a genetic variation of another gene located near the *GSTT1* gene that is related to overall survival in GC patients.

Since, the effect of the *GSTT1* polymorphisms in GC were confirmed by a recent meta-analysis, especially in the Asian population (24), and studies investigating the association between GST polymorphisms and overall survival in GC have been relatively rare, a longitudinal study will be needed, with larger, and ethnically diverse cohorts to confirm the present results.

In summary, *XRCC1* codon 194 Trp presence is correlated with a higher risk of venous invasion in GC patients. Furthermore, the *GSTT1* null genotype is significantly associated with better survival in GC patients. GC may have different biological behavior in individual tumors, and this may lead to various clinical phenotypical prognoses. Furthermore, genetic differences between the patients in the presence of malignant tumor may also affect the outcome. Thus, it may be necessary to place greater emphasis on the heterogeneity within a tumor, as well as host genetic differences. The potential usefulness of *XRCC1* and *GSTT1* polymorphisms as molecular markers for physicians to conduct more appropriate clinical management of GC is demonstrated.

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