Orotate Phosphoribosyltransferase Gene Polymorphism Predicts Toxicity in Patients Treated with Bolus 5-Fluorouracil Regimen

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Abstract

Purpose: We investigated whether the determination of orotate phosphoribosyltransferase (*OPRT*) and thymidylate synthase (*TYMS*) polymorphisms could predict the toxicity of 5-fluorouracil (5-FU) in colorectal cancer patients.

Experimental Design: The determination of OPRT and TYMS genotypes were done in genomic DNA extracted from blood by PCR amplification in 69 patients treated with bolus 5-FU as adjuvant chemotherapy. Associations between these polymorphisms and toxicity were evaluated retrospectively.

Results: The Ala allele in OPRT $Gly^{213}Ala$ polymorphism and the two tandem repeats (2R) in *TYMS* promoter polymorphism were associated with grade 3 to 4 neutropenia and diarrhea. The multivariate logistic regression models revealed that only *TYMS* promoter polymorphism had an independent value to predict grade 3 to 4 neutropenia [odds ratio, 19.2 for patients with the 2R allele compared with patients with homozygous with the three repeat (3R) alleles], whereas both OPRT and TYMS promoter polymorphisms were independent predictive factors for grade 3 to 4 diarrhea (odds ratio, 13.3 for patients with the Ala allele compared with patients in the Gly/Gly genotype and 11.1 for patients with the 2R allele compared with patients in the 3R/3R genotype). A significant difference was observed in the time to onset of severe toxicity, defined as grade 4 neutropenia and/or grade 3 to 4 gastrointestinal toxicities according to *OPRT* and *TYMS* promoter polymorphisms.

Conclusion: OPRT $Gly^{213}Ala$ polymorphism seems to be a useful marker for predicting toxicity to bolus 5-FU chemotherapy. Prospective translational treatment trials including larger number of patients are needed to confirm our results.

Despite the recent development of various new drugs, 5-fluorouracil (5-FU) still plays a major role in chemotherapy for colorectal cancer. Although the drug has been in clinical use for almost 50 years, there is still no clear methodology to identify patients who are likely to benefit most from the treatment. Even with different doses and schedules, the response rate of metastatic colorectal cancer is only 21% when treated with 5-FU and leucovorin (1), with the incidence rates of grade 3 to 4 hematologic toxicity being 31% with 5-FU bolus and 4% with continuous infusion regimens, respectively, and grade 3 to 4 nonhematologic toxicity of 13% to 14% in both regimens (2). Therefore, there is a clear need to identify markers to predict the treatment effect and toxicity to provide a rational basis for treatment selection.

The main mode of action of 5-FU is thought to be the inhibition of thymidylate synthase (TS), an essential DNA synthetic enzyme that catalyses the methylation of dUMP to dTMP, through the binding of fluorodeoxyuridine monophoshate (FdUMP) to TS protein (3). A polymorphic 28-bp tandem repeat in the promoter enhancer lesion (*TSER*), which usually presents as a double-tandem repeat (2R) or a triple-tandem repeat (3R), has been described in the 5′-untranslated region of the TS gene (*TYMS*; ref. 4). This polymorphism may affect the translational efficacy of the gene and predict not only the response (5–8) but also the toxicity (7, 9) when treated by fluoropyrimidine-based chemotherapy. More than 80% of 5-FU given is inactivated by dihydropyrimidine dehydrogenase (DPD) in the liver (10). In patients suffering from severe 5-FU-associated toxicity, several mutations in the DPD gene (*DPYD*) have been identified (11).

Although the importance of the value of TS and DPD in the cytotoxicity of 5-FU is recognized (12), the contribution of phosphorylation is necessary to activate 5-FU into its nucleotides (13). The preferential use of the pathway directly to FUMP by orotate phosphoribosyltransferase (OPRT) was revealed to correlate with the cytotoxicity of 5-FU (13–15). FUMP is then phosphorylated to fluorouridine diphosphate, which can be either converted to FdUMP or phosphorylated to the active metabolite fluorouridine triphosphate (3). Fluorouridine triphosphate is extensively incorporated into RNA (F-RNA), disrupting normal RNA processing and function (3). In a mouse model, 5-FU-induced gastrointestinal toxicity has been reported

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to be due to F-RNA, regardless of FdUMP levels (16). Additionally, potassium oxonate, an inhibitor of OPRT, decreased the levels of FUMP followed by a decrease in F-RNA by ~70% in the small intestine, and there was a reduction in gastrointestinal toxicity (16, 17). Taken together with these results, an association between OPRT expression in normal tissues and 5-FU-induced gastrointestinal toxicity should be considered.

Uridine monophosphate (UMP) synthase is a bifunctional enzyme catalyzing the last two steps of *de novo* pyrimidine biosynthesis, OPRT and orotidine-5′-monophosphate decarboxylase (18). Loss of enzymatic activity results in hereditary orotic aciduria, a rare autosomal recessive disorder (19). Molecular investigation of UMP synthase deficiency in Japanese orotic aciduria patients revealed three rare disease-related single nucleotide polymorphisms of R96G (A-to-G transition; nucleotide 286), G429R (G-to-C transversion; nucleotide 1285), and V109G (T-to-G transversion; nucleotide 326), which compromise OPRT function. In addition, two common non–disease-related polymorphisms, $Gly^{213}Ala$ polymorphism (G-to-C transversion; nucleotide 638) within exon 3 and 440Gpoly within exon 6, were identified. Although 440Gpoly is a silent polymorphism, constructs encoding the $Gly^{213}Ala$ substitution seemed to confer a significant OPRT activity increase in *Sf*21 insect cells using a baculovirus expression system (>150% normal; ref. 19). These data indicate the possibility of predicting of 5-FU-induced toxicity based on *OPRT* $Gly^{213}Ala$ polymorphism in normal tissues.

The aim of this study is to determine whether *OPRT* $Gly^{213}Ala$ polymorphism and *TYMS* promoter polymorphisms may be predictive of toxicity in patients receiving 5-FU adjuvant chemotherapy. We also evaluated *OPRT* mRNA expression and OPRT activity in normal colon mucosa in relation to *OPRT* $Gly^{213}Ala$ polymorphism.

Materials and Methods

Patients. From January 2000 to August 2002, all 69 consecutive patients, who underwent radical surgery and received bolus 5-FU therapy combined with leucovorin as adjuvant chemotherapy in the Department of Digestive and General Surgery of Saitama Medical School, were included. Eligible patients had histologically proven adenocarcinoma of the colon or rectum. Patients were eligible if they had Dukes' B2 with evidence of obstruction, perforation, or invasion of adjacent organs, or Dukes' C tumors, an Eastern Cooperative Oncology Group performance status of ≤1, and adequate bone marrow/renal/hepatic functions. Excluding criteria were any prior or concurrent radiation therapy or chemotherapy. Written informed consent was obtained from all patients to use their blood and tissue samples for research purposes, with the approval of the institute's ethical committee.

This study cohort consisted of 43 men and 26 women, with a median age of 65 years ranging from 36 to 78 years. The performance status was 0 and 1 in 55 and 14 patients, respectively. The primary tumor was located in the colon in 56 cases and in the rectum in 13 cases. The depth of invasion according to the tumor-node-metastasis classification system were 15 T_1 or T_2 tumors, 33 T_3 tumors, and 21 T_4 tumors. There were 6 Dukes' B2 and 63 Dukes' C tumors.

All eligible patients were treated between 14 and 35 days after surgery with the Roswell Park regimen (500 mg/m^2 5-FU i.v. bolus weekly for 6 weeks, given 1 hour after L-leucovorin infusion, combined with 250 mg/m^2 L-leucovorin by 2-hour infusion; four treatment cycles are given, each consisting of six weekly treatments followed by a 2-week rest period).

Toxicity was recorded by grade according to the National Cancer Institute Common Toxicity Criteria version 2.0. Physical examination, a full blood count, and serum chemistry were done before every administration of drugs. Before the start of every injection, the drug dose was reevaluated according to toxicity. If the absolute neutrophil count was <1,500/μL, and if the platelet count was under 75,000/μL, then treatment was delayed until the recovery of bone marrow function. If grade 4 neutropenia or thrombocytopenia, or grade 3 to 4 gastrointestinal toxicities (i.e., diarrhea, stomatitis, nausea, and vomiting) were observed after the previous injection, the 5-FU dose was reduced to 400 mg/m^2 in subsequent courses. Severe toxicity was defined as grade 4 neutropenia or grade 3 to 4 gastrointestinal toxicities or both.

Blood and tissue samples. Blood sampling was done preoperatively in all 69 patients. DNA from peripheral blood mononuclear cells was isolated using a modified QIAamp DNA Blood Maxi kit protocol (Qiagen, Santa Clarita, CA) and was quantified using a PicoGreen dsDNA Quantitation kit (Molecular Probes, Eugene, OR).

In 37 patients who were enrolled initially in this study, we made archival fresh frozen samples of normal colonic mucosa, located apart from the primary tumor, at the time of surgery. Immediately after resection, the normal mucosa was divided into two equal portions of at least 500 mg each. Both portions were fresh frozen in liquid nitrogen until the time of RNA extraction and measurement of OPRT activity.

Genotyping. Samples were genotyped for *OPRT* $Gly^{213}Ala$ polymorphism by Assay-by-Design by Applied Biosystems (ABI, Foster City, CA), as previously described (20). Reactions were done with the following protocol on a GeneAmp PCR 9700 or 7700 ABI Sequence Detection System: 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. A post-PCR plate read on the 7700 was used to determine genotype. Taqman Single Nucleotide Polymorphism Genotyping Assay (Applied Biosystems), prevalidated assay including the specific primer and probes, was used for genotyping (assay ID C_1901447_10, rs1801019).

The genotyping of *TYMS* promoter polymorphisms was carried out by using PCR protocol as described previously (7). Products of 210 bp (2R/2R), 238 bp (3R/3R), or both of products (2R/3R) were observed.

Analysis of OPRT activity and mRNA expression. OPRT activities in frozen samples was measured by the paper disc method (21, 22). Briefly, the tissue samples were homogenized in a 2-fold volume of 50 mmol/L Tris-HCl buffer (pH 7.5) containing 1.5 mmol/L $MgCl_2$ and 2 mmol/L DTT. After centrifuging (105,000 × *g*, 1 hour, 4°C), 200 μL of supernatant were collected and incubated at 37°C with 1.6 μmol/L [^{3}H]5-FU (2.5 nCi), 2 μmol 5-phosphoribosyl 1-pyrophosphate, 6 μmol β-glycerophosphate, and 240 nmol α,β-methylene adenosine 5′-diphosphate in a total volume of 200 μL. Aliquots of the reaction mixture were removed after 5, 10, and 15 minutes of incubation, and the reaction was stopped immediately by placing them in a boiling water bath. After centrifugation, 20 μL of the supernatant were spotted onto anion exchange filter paper disc made from DEAE-cellulose, and the disc was repeatedly washed to remove unreacted [^{3}H]5-FU. The filter paper disc was placed in a scintillation vial followed by the additional 8 mL of Scintisole EX-H (Wako, Tokyo, Japan), and the radioactivity of [^{3}H]FUMP was measured. The OPRT activity (pmol/min per mg protein) was calculated based on the amount of FUMP produced, which was proportional to the radioactivity, and the protein concentration in the enzyme solution was measured by the method of Lowry et al. (23).

Total RNA for each sample was isolated using the RNeasy mini kit (Qiagen, Inc., Chatsworth, CA) followed by cDNA synthesis as previously described (15). Quantitation of cDNA of the *OPRT* gene and an internal reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was done by a fluorescence based real-time detection method (Taqman), as described previously. The PCR reaction mixture consisted of 600 nmol/L of each primer; 200 nmol/L probe; 2.5 units AmpliTaq Gold Polymerase; 200 μmol/L each dATP, dCTP, and dGTP; 400 μmol/L dTTP; 5.5 mmol/L $MgCl_2$, and 1× Taqman Buffer A containing a reference dye, to a final volume of 25 μL (all reagents from

Applied Biosystems). Primer and probe sequences were described in a previous report (15). Cycling conditions were 50°C for 10 seconds and 95°C for 10 minutes followed by 46 cycles at 95°C for 15 seconds and 60°C for 1 minute.

Quantification was prepared using the relative standard curve method (24). The standard curve was created automatically by the ABI PRISM 7700 Sequence Detection System by plotting the threshold cycle (C_T) against each input amount (containing 16, 4, 1, 0.25, 0.063, 0.016, 0.0039 ng) of control total RNA (total starting RNA), prepared from MDA-MB-231 human breast tumor cells (American Type Culture Collection, Manassas, VA; ref. 25). The coefficient of linear regression for each standard curve was >0.990. For each unknown sample, the relative amount was calculated using linear regression analysis from the respective standard curve. Taqman analyses yield values that are expressed as ratios between two absolute measurements (gene of interest/internal reference gene).

Statistical analysis. The χ^2 test was used to compare the observed genotype distribution with that expected by the Hardy-Weinberg equilibrium. To test for an association of observed genotype with clinicopathologic features, the χ^2 test or Fisher's two-tailed exact test were done. The association between the genotype and toxicity to chemotherapy was evaluated by the nonparametric Kruskal-Wallis test. In univariate and multivariate analysis, the relation with toxicities was examined with logistic regression analysis, and the odds ratio (OR) were calculated. The five valuables included in the multivariate logistic regression model were chosen among factors related to patient characteristics (age, gender, and performance status) and polymorphisms of *TYMS* promoter lesion and *OPRT* Gly^{213}Ala. The Kaplan-Meier method and the log-rank test were adopted to estimate and compare cumulative incidences of severe toxicity. The Kruskal-Wallis test or the Mann-Whitney *U* test was used to assess the association between genotype and OPRT activity or mRNA level. The Spearman correlation was used to evaluate the association between OPRT activity and mRNA level. $P < 0.05$ was taken to indicate a statistically significant difference. Statistical analysis was done using JMP software version 5.1 (SAS Institute, Inc., Cary, NC).

Results

Distributions of OPRT Gly^{213} Ala polymorphism and TYMS promoter polymorphism. *OPRT* Gly^{213}Ala polymorphism was successfully assessed for all 69 patients. The genotype TYMS promoter polymorphisms were obtained for 65 patients because of missing four samples. The distribution of the Gly^{213}Ala genotype was 8.7% Ala/Ala [6 patients; 95% confidence interval (95% CI), 2.0-15.3%], 37.7% Ala/Gly (26 patients; 95% CI, 26.2-49.1%), and 53.6% Gly/Gly (37 patients; 95% CI, 41.9-65.4%). The frequency of the rare allele (Ala) was 27.5%. The distribution is in close agreement with that predicted by the Hardy-Weinberg equilibrium. The distribution of the *TYMS* promoter polymorphism was 4.6% 2R/2R (3 patients; 95% CI, 0.1-4.7%), 16.9% 2R/3R (11 patients; 95% CI, 13.3-20.6%), and 78.5% 3R/3R (51 patients; 95% CI, 74.8-82.1%). The frequencies of the 2R and 3R allele were 13.1% and 86.9%, respectively.

There was no statistical association of *OPRT* Gly^{213}Ala and *TYMS* promoter polymorphisms with the clinicopathologic features, such as age, gender, performance status, location of primary tumor (colon, rectum), tumor depth of invasion, and Dukes' classification (data not shown).

Analysis of toxicity in terms of genotypes. The most prevalent toxicities reported were neutropenia seen in 10.1% (6 patients with grade 3 and 1 patient with grade 4) and diarrhea in 15.9% (10 patients with grade 3 and 1 patient with grade 4) of all patients. Severe toxicity, defined as grade 4 neutropenia or grade 3 to 4 gastrointestinal toxicities or both, was observed in 11 of all 69 patients: one patient experienced grade 4 neutropenia and diarrhea, and two patients experienced grade 3 diarrhea and nausea or vomiting. No toxic deaths were observed in this study.

Grade 3 to 4 neutropenia was observed in 2.7% (1 of 37) of patients in the Gly/Gly group compared with 15.4% (4 of 26) in the Ala/Gly group and 33.3% (2 of 6) in the Ala/Ala group (Kruskal-Wallis test, $P = 0.0393$; Table 1). There was grade 3 to 4 neutropenia in 66.7% (2 of 3) of patients with the 2R/2R genotype compared with 27.2% (3 of 11) in the 2R/3R group and 3.9% (2 of 51) in the 3R/3R group (Kruskal-Wallis test, $P = 0.0005$; Table 1). There was grade 3 to 4 diarrhea in all six patients with the Ala/Ala genotype compared with 15.4% (4 of 26) in the Ala/Gly group and 2.7% (1 of 37) in the Gly/Gly group (Kruskal-Wallis test, $P < 0.0001$; Table 1). Grade 3 to 4 diarrhea was observed in 66.7% (2 of 3) of patients in the 2R/2R group compared with 36.4% (4 of 11) in the 2R/3R group and 9.8% (5 of 51) in the 3R/3R group ($P = 0.0070$, Kruskal-Wallis test; Table 1).

All six patients in the Ala/Ala genotype experienced severe toxicity, whereas 4 of 26 patients in the Ala/Gly genotype and 1 of 36 patients in the Gly/Gly genotype did ($P < 0.0001$, Kruskal-Wallis test; Table 1). There was severe toxicity observed in 2 of 3 patients with 2R/2R genotype compared with 4 of 11 patients in the2R/3R group and 5 of 51 patients in the 3R/3R group ($P = 0.0005$, Kruskal-Wallis test; Table 1). An OR of 16.4 (95% CI, 2.0-136.7) was observed for patients with the Ala allele compared with the group of patients homozygous for the Gly allele, whereas an OR of 6.9 (95% CI, 1.7-28.1) was observed for patients with the 2R allele compared with the group of patients homozygous for the 3R allele.

Using multivariate analysis, only *TYMS* promoter polymorphism (2R/2R < 2R/3R, 3R/3R) was found to be a statistically significant risk factor for grade 3 to 4 neutropenia, with an OR of 19.2 (95% CI, 2.2-334.4; $P = 0.016$; Table 2). Both *OPRT* Gly^{213}Ala polymorphism (OR, 13.3; 95% CI, 1.9-280.9; $P = 0.026$) and *TYMS* promoter polymorphism (OR, 11.1; 95% CI, 1.6-117.0; $P = 0.022$) were independent variables associated with grade 3 to 4 diarrhea.

In addition, a significant difference was observed in the time to onset of severe toxicity according to *OPRT* Gly^{213}Ala and *TYMS* promoter polymorphisms ($P < 0.0001$ and $P = 0.0009$, respectively, log-rank test; Fig. 1). The median time to onset of severe toxicity was 3 weeks (range, 1-5 weeks) in six patients with the Ala/Ala genotype and 6 weeks (range, 3-13 weeks) in 26 patients with the Ala/Gly genotype. Only one patient with the Gly/Gly genotype experienced severe toxicity, at 8 weeks from the start of chemotherapy.

Among 11 patients with severe toxicity, one patient with the Ala/Ala *OPRT* polymorphism and the 2R/2R *TYMS* polymorphism needed to be hospitalized due to coincidentally occurring grade 4 neutropenia and diarrhea, and the treatment was stopped. 5-FU-based chemotherapy was successfully following by the treatment postponement and the dose reduction in four patients (three patients with the Ala/Gly genotype and one patient with the Gly/Gly genotype), whereas treatment was halted in the remaining six patients (three patients with the Ala/Ala genotype and three patients with Ala/Gly genotype) because of severe toxicity, although we attempted dose modification.

OPRT activity and mRNA expression in relation to Gly^{213} Ala variants. In 37 patients who were enrolled initially in this

Table 1. Association between *OPRT* Gly^{213}Ala and *TYMS* promoter genotypes and the occurrence of toxicities

	OPRT Gly^{213}Ala genotypes				*TYMS* promoter genotypes			
	Ala/Ala	Ala/Gly	Gly/Gly	*P*	2R/2R	2R/3R	3R/3R	*P*
No. patients (%)	6 (8.7%)	26 (37.7%)	37 (53.6%)		3 (4.6%)	11 (16.9%)	51 (78.5%)	
Neutropenia (no. patients)								
Grade 0-2	4	21	36	0.0393*	1	8	49	0.0005*
Grade 3	1	4	1		1	3	2	
Grade 4	1				1			
Diarrhea (no. patients)								
Grade 0-2		22	36	<0.0001*	1	7	49	0.0070*
Grade 3	5	4	1		1	4	5	
Grade 4	1				1			
Severe toxicity†								
Not experienced		22	36	<0.0001	1	7	46	0.0003
Experienced	6	4	1		2	4	5	

NOTE: Toxicities were graded according to version 2.0 of the National Cancer Institute Common Toxicity Criteria. *P* as calculated with the nonparametric Kruskal-Wallis test.

**P* with regard to toxicity comparing grade 0 to 2 versus grade 3 to 4.

† Grade 4 neutropenia and/or Grade 3 to 4 gastrointestinal toxicities.

study, both *OPRT* mRNA expression and OPRT activity were measured in normal colon mucosa. The distribution of the Gly^{213}Ala genotype in these 37 patients was 8.1% in Ala/Ala (3 patients; 95% CI, 0.0-16.9%), 29.7% in Ala/Gly (11 patients; 95% CI, 15.0-44.5%), and 62.2% in Gly/Gly (23 patients; 95% CI, 46.5-77.8%), which is similar in all 69 patients.

We examined the relationship between *OPRT* mRNA expression and the *OPRT* Gly^{213}Ala polymorphism. The median gene expressions in patients in the Ala/Ala group and the Ala/Gly group were 1.62 and 0.87, respectively, whereas the median gene expression in patients in the Gly/Gly group was 0.53 (Kruskal-Wallis test, $P < 0.0001$; Fig. 2).

Analysis of the Gly^{213}Ala polymorphism with respect to OPRT activity revealed a significant association between the genotype and OPRT activity in normal colon mucosa. The median OPRT activity in patients with the Ala/Ala genotype and the Ala/Gly genotype were 0.26 and 0.13 pmol/min per mg protein, respectively, whereas the median OPRT activity in patients with

Table 2. Association between patient characteristics and polymorphisms in *OPRT* Gly^{213}Ala and *TYMS* promoter and grade 3 to 4 neutropenia and diarrhea by logistics analysis

Variables	No. patients	Grade 3 to 4 neutropenia				Grade 3 to 4 diarrhea			
		Univariate		Multivariate		Univariate		Multivariate	
		OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age									
<70	37	1	0.990	1	0.704	1	0.861	1	0.704
≥70	28	1.0 (0.2, 4.9)		1.5 (0.2, 16.1)		1.1 (0.3, 4.2)		1.4 (0.3, 8.8)	
Gender									
Male	39	1	0.336	1	0.646	1	0.787	1	0.101
Female	26	2.2 (0.4, 12.0)		0.6 (0.1, 4.8)		0.8 (0.2, 3.1)		0.2 (0.0, 1.1)	
Performance status									
0	52	1	0.127	1	0.148	1	0.511	1	0.743
1	13	3.6 (0.7, 19.0)		4.5 (0.6, 50.2)		1.7 (0.3, 7.0)		1.3 (0.2, 7.7)	
OPRT Gly^{213}Ala									
Gly/Gly	32	1	0.072	1	0.239	1	0.014	1	0.026
Ala/Gly, Ala/Ala	33	7.4 (1.2, 144.1)		4.5 (0.5, 111.9)		14.5 (2.5, 276.7)		13.3 (1.9, 280.9)	
TYMS promoter									
3R/3R	51	1	0.003	1	0.016	1	0.007	1	0.022
2R/3R, 2R/2R	14	13.6 (2.5, 106.1)		19.2 (2.2, 334.4)		6.9 (1.7, 29.8)		11.1 (1.6, 117.0)	

NOTE: Multivariate analysis was done by using the patient characteristics (age, gender, and performance status), *OPRT* Gly^{213}Ala genotype, and *TYMS* promoter genotype.

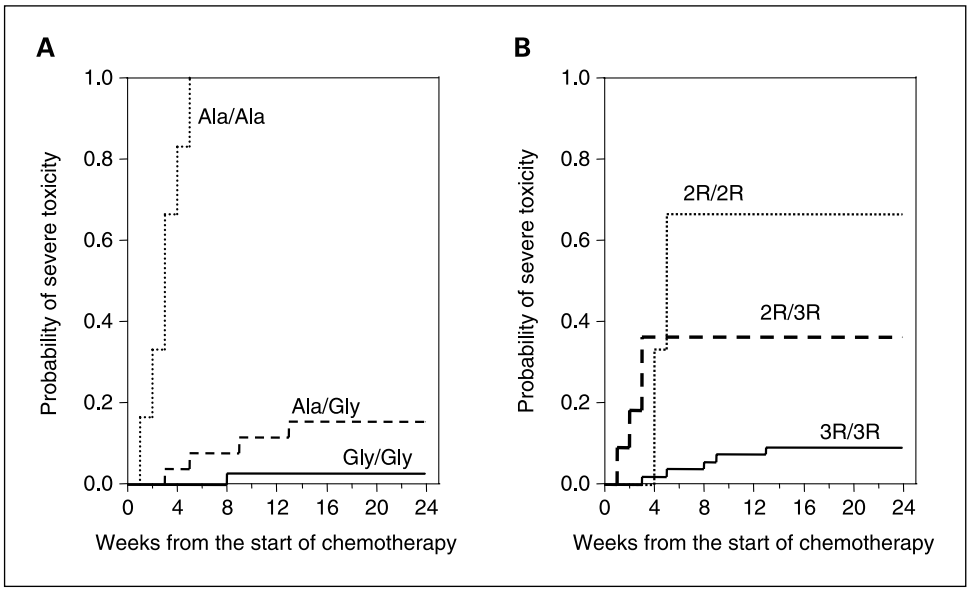

Fig. 1. Cumulative incidence of severe toxicity according to *OPRT* Gly^{213}Ala polymorphism and *TYMS* promoter polymorphism. *A, OPRT* Gly^{213}Ala polymorphism ($P < 0.0001$, log-rank test). *Solid line,* Gly/Gly genotype ($n = 37$); *broken line,* Ala/Gly genotype ($n = 26$); *dotted line,* Ala/Ala genotype ($n = 6$). *B, TYMS* promoter polymorphism ($P = 0.0009$, log-rank test). *Solid line,* 3R/3R genotype ($n = 51$); *broken line,* 2R/3R genotype ($n = 11$); *dotted line,* 2R/2R genotype ($n = 3$).

the Gly/Gly genotype was 0.07 (Kruskal-Wallis test, $P = 0.0002$; Fig. 2). The Ala allele showed a significant association with higher OPRT activity (median, 0.13 and 0.07 pmol/min per mg protein for patients with the Ala allele and those with the Gly/Gly genotype, respectively; Mann-Whitney U test, $P < 0.0001$).

There was a positive correlation between *OPRT* mRNA level and OPRT activity in normal colon mucosa (Spearman rank correlation coefficient 0.71, $P < 0.0001$).

Discussion

In this study, we showed that *OPRT* Gly^{213}Ala polymorphism could help in predicting toxicity of grade 3 or 4 neutropenia and diarrhea among colorectal cancer patients who received 5-FU adjuvant chemotherapy. Especially, *OPRT* Gly^{213}Ala and *TYMS* promoter polymorphisms were independent factors to predict grade 3 to 4 diarrhea, whereas only *TYMS* promoter polymorphism had the independently predictive valuable for grade 3 to 4 neutropenia.

There is ample evidence that a deficiency of DPD is associated with severe toxicity after the administration of 5-FU. In patients who are deficient for DPD, 5-FU clearance is dramatically reduced and standard doses of 5-FU cause excessive toxicity in these patients (10, 26). DPD deficiency is caused at least in part by *DPYD* polymorphisms that result in complete or partial loss of DPD activity (11, 27, 28). To date, the most common consistent data are for allele *DPYD**2, which is a splice site mutation (G to A) that causes skipping of exon 14 (IVS14+1G>A), resulting in the production of a truncated mRNA and the formation of a defective protein (27). This defective protein degrades rapidly followed by decreased detectable DPD activity (28). This mutation was commonly

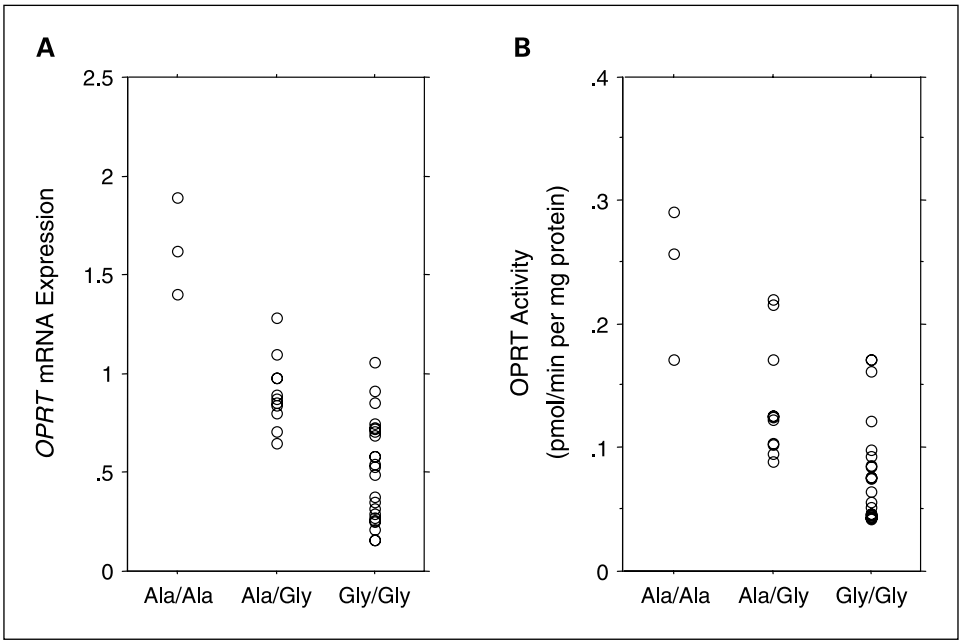

Fig. 2. Association of *OPRT* mRNA expression and activity in normal colon mucosa with *OPRT* Gly^{213}Ala polymorphism ($n = 37$). *A, OPRT* mRNA expression (Kruskal-Wallis test, $P < 0.0001$). *B,* OPRT activity (Kruskal-Wallis test, $P = 0.0002$).

detected in 24% to 28% of all patients suffering from severe 5-FU toxicity, whereas the frequency of the mutant allele is actually 0% in the Japanese population (11, 29, 30). These results show that this mutation might be justified for screening for DPD deficiency, not predicting toxicity before 5-FU-based chemotherapy. Thus, we did not perform the genotyping of *DPYD**2 in this study.

Unlike in DPYD, the significant inverse association between the number of the 28-bp tandem repeats in *TSER* and severe toxicity to 5-FU is relevant. Patients with homozygous 2R/2R genotype experienced severe toxicity (grade 3) with an incidence of 63% compared with 32% in the group 2R/3R and 27% in the group 3R/3R, when treated with 5-FU-based chemotherapy for metastatic colorectal cancer (7). Subsequently, an OR of 20 has been reported for patients with the homozygous genotype carrying two 2R alleles compared with the reference group of patients homozygous for the 3R alleles in *TSER*, when treated with adjuvant or palliative 5-FU-based chemotherapy (9). We confirmed the association between *TYMS* polymorphism and severe toxicity to 5-FU, even in the Japanese population. The frequency of the 2R allele has been reported to be 19% in the Japanese population, which is lower than the 40% among Caucasian population (31).

There is little knowledge about the relation between the toxicity and polymorphism in genes responsible for 5-FU activation. We showed for the first time that *OPRT* $Gly^{213}Ala$ polymorphism could help in predicting toxicity, especially grade 3 to 4 diarrhea, among colorectal cancer patients who received 5-FU chemotherapy. Patients with the Ala allele were 16 times more likely to have severe toxicity compared with those with the Gly/Gly genotype. Moreover, a conspicuous finding was that the onset of severe toxicity occurred earlier from the start of chemotherapy in patients with the Ala/Ala genotype than those with other genotypes. To the best of our knowledge, the positive association between genotype and the onset of toxicity has not been previously reported. *OPRT* $Gly^{213}Ala$ polymorphism might predict not only the risk of toxicity but also the time of the occurrence of severe toxicity.

In our population with colorectal cancer, the frequency of the Ala allele of *OPRT* $Gly^{213}Ala$ polymorphism was 27.5%, quite similar to the 26% previously reported in the Japanese healthy population (19). Of note, albeit based on a small number of patients, *OPRT* $Gly^{213}Ala$ polymorphism statistically correlates with OPRT activity in normal colon mucosa. This result is concordant with the *in vitro* study (19). The Ala allele was also associated with increased *OPRT* mRNA level in normal tissue, leading to the hypothesis that this polymorphism could play a role in mRNA stability and translation. The mechanism of induction of gastrointestinal toxicity was reported to be due to the incorporation of F-RNA, not the inhibition of the biosynthesis of dTMP through conversion of 5-FU to FdUMP (16). Hence, higher OPRT activity, leading to elevate the level of F-RNA in small intestine after the administration of 5-FU, could be reasonably expected to increase the likelihood of severe diarrhea in patients with the Ala allele.

In conclusion, *OPRT* $Gly^{213}Ala$ polymorphism seems to be a significant predictor of 5-FU-induced toxicity in patients treated with bolus 5-FU adjuvant therapy. *OPRT* $Gly^{213}Ala$ polymorphism had an independent value only for prediction of severe diarrhea, whereas *TYMS* promoter polymorphism was an independent factor to predict both severe neutropenia and diarrhea. These findings could generate new questions concerning the correlation of *OPRT* $Gly^{213}Ala$ and *TYMS* polymorphisms and the predictive value of combining the information from two polymorphisms. At present, we cannot answer the questions because the current study was a limited retrospective one involving a relatively small number of patients. Prospective translational treatment trials, including larger number of patients, are needed to corroborate our results and resolve these questions.

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