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Impact of *DPYD*, *DPYS*, and *UPB1* gene variations on severe drug-related toxicity in patients with cancer

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Abstract

Cancer treatment with a fluoropyrimidine (FP) is often accompanied by severe toxicity that may be dependent on the activity of catalytic enzymes encoded by the *DPYD*, *DPYS*, and *UPB1* genes. Genotype-guided dose individualization of FP therapy has been proposed in western countries, but our knowledge of the relevant genetic variants in East Asian populations is presently limited. To investigate the association between these genetic variations and FP-related high toxicity in a Japanese population, we obtained blood samples from 301 patients who received this chemotherapy and sequenced the coding exons and flanking intron regions of their *DPYD*, *DPYS*, and *UPB1* genes. In total, 24 single nucleotide variants (15 in *DPYD*, 7 in *DPYS* and 2 in *UPB1*) were identified including 3 novel variants in *DPYD* and 1 novel variant in *DPYS*. We did not find a significant association between FP-related high toxicity and each of these individual variants, although a certain trend toward significance was observed for p.Arg181Trp and p.Gln334Arg in *DPYS* ($P = .0813$ and .087). When we focused on 7 *DPYD* rare variants (p.Ser199Asn, p.Ile245Phe, p.Thr305Lys, p.Glu386Ter, p.Ser556Arg, p.Ala571Asp, p.Trp621Cys) which have an allele frequency of less than 0.01% in the Japanese population and are predicted to be loss-of-function mutations by in silico analysis, the group of patients who were heterozygous carriers of at least one these rare variants showed a strong association with FP-related high toxicity ($P = .003$). Although the availability of screening of these rare loss-of-function variants is still unknown, our data provide useful information that may help to alleviate FP-related toxicity in Japanese patients with cancer.

KEYWORDS

5-fluorouracil, *DPYD*, *DPYS*, fluoropyrimidine, *UPB1*

Abbreviations: 5FU, 5-fluorouracil; CDDP, cisplatin; CPT-11, irinotecan hydrochloride hydrate; CTCAE, Common Terminology Criteria for Adverse Events; DHP, dihydropyrimidinase; DPD, dihydropyrimidine dehydrogenase; DTX, docetaxel hydrate; FP, fluoropyrimidine; GEM, gemcitabine hydrochloride; Jmorp, Japanese Multi Omics Reference Panel; L-OHP, oxaliplatin; PTX, paclitaxel; SIFT, Sorting Intolerant From Tolerant; βUP, β-ureidopropionase.

1 | INTRODUCTION

Fluoropyrimidines including 5 5FU and its prodrugs are widely used in the treatment of various malignancies including head and neck, gastrointestinal, or breast cancers.[1-3] FPs have a narrow therapeutic index and up to 30% of treated patients develop early-onset severe toxicity such as diarrhea, nausea, mucositis, stomatitis, myelosuppression, neurotoxicity, and hand-foot syndrome.[4-6] FP toxicity is largely dependent on its catabolism. Most FP molecules are inactivated by DPD and FP-related toxicity is often caused by an inherited reduced activity of this enzyme.[7-9] Patients with a DPD deficiency have an increased risk of developing severe treatment-related toxicity from a standard dose of FP.[10] A partial DPD deficiency is present in 3%-5% of the North American and European general population.

The DPD gene, *DPYD*, is located on chromosome 1p21 and is comprised of 23 exons.[11] The 4 *DPYD* variants considered most clinically relevant with statistically significant associations with severe toxicity are c.1905 + 1G>A (*DPYD**2A, rs3918290, IVS14 + 1G>A), c.2846A>T (rs67376798, D949V), c.1679T>G (rs55886062, *DPYD*13, I560S) and c.1236G>A (rs56038477, E412E, in haplotype B3).[12,13] Hence, *DPYD* genotype-guided dose individualization of FP therapy has now been conducted in some western countries.[10] However, to our knowledge it has not been performed yet in Japan, possibly because none of these *DPYD* variants have been identified in the Asian population.[14] Recently, 21 *DPYD* allelic variants were identified in 1070 healthy Japanese individuals.[15] The functional alterations caused by these variants were analyzed in vitro and their enzyme activities were characterized.[14] However, there has been no report to date on the clinical relevance of *DPYD* variants as predictors of FP-associated toxicity in Japanese people.

It is thought that decreased activity of the enzymes DHP and βUP, which are located downstream of DPD in FP catabolism, may also play a role in FP-associated toxicity.[16] The DHP-encoding gene, *DPYS*, is located on chromosome 8q22,[17] and the βUP-encoding gene, *UPB1*, is located on chromosome 22q11.[18] A relationship between *DPYS* and *UPB1* gene variations and severe FP-related toxicity has been reported,[16] but no other data currently support this association, which thus remains to be fully elucidated. It is known that the Japanese prevalence of βUP deficiency is relatively high (1 per 6000 newborns).[19] We have also reported that some *DPYS* variants may be more common than expected in East Asian groups.[20] These findings have prompted us to screen for variants of the genes associated with FP-related toxicity in Japanese subjects.

We have here evaluated the association between *DPYD*, *DPYS*, and *UPB1* gene variations and severe FP-related toxicity in Japanese patients with cancer. This is the first report to assess the clinical relevance of *DPYD*, *DPYS*, and *UPB1* variants as predictors of severe FP-associated toxicity in East Asians.

2 | MATERIALS AND METHODS

2.1 | Patients and sample collection

Blood samples of 301 consenting patients who received or were receiving FP-based chemotherapy were collected between 2018 and 2020. All patients were of East Asian origin. These 301 patients were recruited at Fujita Health University. All treatments, patient characteristics, concurrent therapy and adverse effects (gastrointestinal [nausea, vomiting, diarrhea, oral mucositis], neutropenia, hand-foot syndrome, acute kidney injury) developed within the first 2 cycles of treatment in this cohort and were classified according to the CTCAE v4.0. We divided these subjects into 2 groups in accordance with the grade of toxicity for statistical purposes. The high-toxicity group included patients who experienced severe toxicity presenting with CTCAE grade 3-5 adverse events in any category. The low-toxicity group included patients who experienced low toxicity involving CTCAE grade 0-2 adverse events.

2.2 | DPYD, DPYS, UPB1 sequencing analysis

Genomic DNA was extracted from aliquots of the study patient blood specimens using a standard procedure. We designed a custom AmpliSeq panel for the sequencing of coding exons and flanking intron regions (± 10 bp) of *DPYD*, *DPYS*, and *UPB1*. Library preparation for amplicon sequencing was performed using AmpliSeq Library PLUS for Illumina. Libraries were sequenced on the MiSeq platform with 150 bp paired-end reads (Illumina, San Diego, CA). Sequencing data were analyzed with Illumina Basespace DNA Amplicon App. We used the UCSC genome browser (http://genome-asia.ucsc.edu/human GRCh37/hg19) as the human genome assembly. Illumina Variant Studio was used for annotation and filtration of genomic variants with a Pass Filter read depth > 50×. Allele frequency was investigated with gnomAD browser beta (http://gnomad.broadinstitute.org/) and Jmorp https://jmorp.megabank.tohoku.ac.jp).

The in silico analysis of each variant was performed using Polymorphism Phenotyping ver. 2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2) and SIFT (http://sift-dna.org) to predict the functional impact on the protein product. In the PolyPhen-2 program, the investigated variant is categorized as probably damaging (probability score > 0.85), possibly damaging (probability score between 0.16 and 0.85), or benign (probability score less than or equal to 0.15). SIFT is a tool for sorting intolerant from tolerant amino acids. The evaluated amino acid substitution is predicted as damaging if the score is <.05 and is predicted to be tolerated if the score is greater than or equal to .05.

2.3 | Statistical analyses

The study patient characteristics were presented using median and range for continuous variables, and frequencies and proportions for categorical variables. The Fisher exact test was used to identify the association of

DPYD, *DPYS*, and *UPB1* variations with FP-related high toxicity because some categories would have an expected count of 5 or less. The frequencies of high-toxicity and low-toxicity groups were compared between each genotype. Due to the exploratory nature of this study, a *P*-value less than .05 was considered statistically significant, and $.05 < P < .1$ was recognized as indicating a certain trend toward significance. Statistical analyses were conducted using R software version 3.6.2 (www.r-project.org).

3 | RESULTS

3.1 | Patient characteristics

The age, treatments, and cancer types of the 301 patients are listed in Table 1. The most commonly used regimen in this cohort was 5FU + L-OHP/CPT-11 + α(molecular target) (54.5%, n = 164), followed by 5FU monotherapy (28.9%, n = 87). A majority of patients (68.1%) had a colorectal tumor (n = 205). During the first 2 cycles of chemotherapy, 18.3% (n = 55) of the patients developed high-toxicity responses (CTCAE grade 3-4), and 81.7% (n = 246) showed low toxicity (CTCAE grade 0-2). No patient in our current series developed a grade 5 adverse event. The most frequent adverse event category observed in the high-toxicity group was neutropenia, followed by gastrointestinal-related issues (Table 2).

Toxicity rates varied and significantly depended on the FP regimen (Table 1). 80% (n = 4/5) and 53.8% (n = 7/13) of patients who received DTX + CDDP+5FU and FOLFOXIRI + α, respectively, developed high toxicity, while, only 1 patient (1.8%) showed high toxicity in 5FU monotherapy.

3.2 | Variant analysis of the *DPYD*, *DPYS*, and *UPB1* genes

In total, 24 non-synonymous single nucleotide variants (15 *DPYD*, 7 *DPYS* and 2 *UPB1*) were identified in our present study population including 3 novel variants in *DPYD* and 1 novel variant in *DPYS*. (Table 3) Seven *DPYD* variants and 2 *DPYS* variants were rare variants with a minor allele frequency in the Japanese population of less than 0.01% (Jmorp). (Table 3) Out of the 24 variants identified, we excluded 1 *DPYD* nonsense variant and one *DPYS* noncoding variant and analyzed the remaining 22 by PolyPhen-2 and SIFT. The results indicated that 18 variants, including all of the rare variants, were predicted to be probably damaging by PolyPhen-2 and/or damaging by SIFT (Table 3). The number of heterozygous and homozygous individuals in the high-toxicity and low-toxicity groups for each of the 24 variants is shown in Table S1.

3.3 | Association of *DPYD*, *DPYS*, and *UPB1* variations with FP-related high toxicity

The *P*-values for each of the variants in relation to an association with FP-related high toxicity are shown in Table 4. In the single

TABLE 1 Baseline characteristics of the study population: n = 301

	Total sample	Low toxicity (grade 0-2) n (%)	High toxicity (grade 3-4) n (%)
Total	301	246	55
Age			
Median	67	66	68
Range	22-85	25-85	22-81
Sex			
Male	179	155 (86.6%)	24 (13.4%)
Female	122	91 (74.6%)	31 (25.4%)
Tumor			
Stomach	70	59 (84.3%)	11 (15.7%)
Colorectal	205	167 (81.5%)	38 (18.5%)
Other tumors	26	20 (76.9%)	6 (23.1%)
5FU + CDDP	17	15 (88.2%)	2 (11.8%)
5FU + L-OHP/CPT-11 + α(molecular target)	164	127 (77.4%)	37 (22.6%)
5FU mono	87	86 (98.9%)	1 (1.1%)
DTX + CDDP+5FU	5	1 (20%)	4 (80%)
FOLFOXIRI + α	13	6 (46.2%)	7 (53.8%)
5FU + PTX	1	1 (100%)	0 (0%)
5FU + GEM/DTX	14	10 (71.4%)	4 (28.6%)

Abbreviations: 5FU, 5 fluorouracil; CDDP, cisplatin; L-OHP, oxaliplatin; CPT-11, irinotecan hydrochloride hydrate; DTX, docetaxel hydrate; PTX, paclitaxel; GEM, gemcitabine hydrochloride.

TABLE 2 Numbers and proportions (%) of patients experiencing different categories of toxicity during the first 2 therapy cycles

Toxicity category	Total sample (n = 301) n (%)	Low toxicity (grade 1-2) (n = 246) n (%)	High toxicity (grade 3-4) (n = 55) n (%)
Gastrointestinal			
Nausea	61 (20.3%)	58 (23.6%)	3 (5.5%)
Vomiting	21 (7%)	18 (7.3%)	3 (5.5%)
Diarrhea	43 (14.3%)	36 (14.6%)	7 (12.7%)
Oral Mucositis	29 (9.6%)	28 (11.4%)	1 (1.8%)
Neutropenia	125 (41.5%)	82 (33.3%)	43 (78.2%)
Hand-foot syndrome	30 (10%)	30 (12.2%)	0 (0%)
Acute kidney injury	10 (3.3%)	10 (4.1%)	0 (0%)

variant analysis, in which we analyzed each variant individually, we did not find any significant association with high toxicity, although a certain trend toward significance was observed for p.Arg181Trp and p.Gln334Arg of *DPYS* ($P = .081$ and .087). *DPYS* p.Arg181Trp was a common variant and was heterozygous in 14 patients who were all classified as low-toxicity group cases. In contrast, heterozygosity for *DPYS* p.Gln334Arg was observed in only 3 patients, 2 of whom developed high toxicity (Table 4). Clinical and genetic information for these 2 heterozygous *DPYS* p.Gln334Arg patients with high toxicity are provided in Table 5. Both patients received 5FU + L-OHP and developed grade 3 neutropenia. In addition, 1 patient presented with grade 1 vomiting and the other presented with grade 2 nausea. Although both patients were simultaneous carriers of other variants including *DPYD* p.Arg29Cys, *DPYD* p.Ile543Val and *DPYS* c.-1T>C, these additional variants are common benign variants found in the total cohort with an allele frequency of 96%, 27% and 68%, respectively. Hence, our results suggested that p.Gln334Arg may contribute to the susceptibility to severe FP-related toxicity.

We next focused on 7 rare *DPYD* variants that have an allele frequency in the Japanese population of less than 0.01%. Six show loss-of-function by in silico analysis ie probably damaging by PolyPhen-2 and/or deleterious by SIFT (p.Ser199Asn, p.Ile245Phe, p.Thr305Lys, p.Ser556Arg, p.Ala571Asp, p.Trp621Cys), and one is a nonsense mutation (p.Glu386Ter). We divided our patients into a rare pathogenic *DPYD* variant group consisting of individuals heterozygous for these 7 rare variants (n = 7), and a group of all other individuals without these rare variants (n = 294). Using the Fisher exact test, we found that the rare pathogenic *DPYD* variant group showed a significant association with FP-related high toxicity ($P = .003$; Table 4). Detail information on the 7 patients in the rare *DPYD* variant group is presented in Table 6. Although these 7 patients also carried other variants (*DPYD* p.Arg29Cys, *DPYD* p.Met166Val, *DPYD* p.Ile543Val, *DPYD* p.Thr768Lys and *DPYS* c.-1T>C), these additional variants showed a frequency of more than 1% and appeared benign. In the rare *DPYD* variant group also, 1 patient carrying a heterozygous *DPYD* p.Ala571Asp variant received 5FU monotherapy which is known as a more tolerable chemotherapy protocol, but developed severe toxicity including grade 3 nausea, grade 3 diarrhea, and grade 1 neutropenia.

4 | DISCUSSION

More than 450 *DPYD* variants have been identified to date as a cause of 5FU-related toxicity in patients with cancer.[14] In the context of 5FU, 4 *DPYD* variants identified in the White population are known to have an impact on enzyme function and FP-related toxicity risk.[21] However, none of these *DPYD* variants has been identified to date in an Asian population.[22] Prospective *DPYD* genotyping has thus proved feasible and effective in White but not in Japanese cases. In our present study, we revealed that *DPYD* nonsynonymous variants with allele frequencies of less than 0.01% in the Japanese population, and with an in silico analysis prediction of loss of function, may be associated with severe FP-related toxicity. Our data lend support to the concept that *DPYD* variants exist also in East Asian populations that affect the enzymatic activity of the protein product and thereby the severity of FP-related toxicity. In this study, we found 7 rare *DPYD* variants that were not found in Japanese genome variation databases. For Japanese allele frequency, we used the Jmorp database, which is based on the data of approximately 4000 Japanese individuals mainly living in the northeastern area of Japan. Since our 301 patients were recruited at our hospital at the central area of Japan, the discordance might be due to regional difference in the allele frequency.

The aim of our present study was the establishment of *DPYD* genotype-guided dose individualization of FP therapy in Japanese patients with cancer that have been performed in some western countries. However, we could not find a specific common variant in our present Japanese cohort that was highly associated with FP-related high toxicity. Sequencing of all coding DNA in the *DPYD* gene has some advantages in relation to screening high-risk individuals for severe FP-related toxicity, although it would not seem reasonable to reduce an FP treatment dose based on insufficient in silico findings. It may therefore be difficult to introduce *DPYD* genotyping as useful prospective screening in Japan. Previously, analysis of DPD enzyme activity has been proposed to be the most reliable method for identifying at-risk patients.[23] For the interpretation of a novel or very rare *DPYD* variant, it is useful to measure the DPD activity in the individuals who carry the variants.

TABLE 3 Information and *P*-values for the variants examined in this study

Genotype	dbSNP	In silico function (PolyPhen-2)	In silico function (SIFT)	Allele frequency (%) (Japanese/east Asian/Total)	*P*-value
DPYD					
NM_000110.3:c.85C>T NP_000101.2:p.Arg29Cys	rs1801265	Benign (0)	Tolerated (0.18)	96.85/92.8/76.6	.507
NM_000110.3:c.496A>G NP_000101.2:p.Met166Val	rs2297595	Probably damaging (1)	Tolerated (0.07)	2.18/1.524/8.585	.146
NM_000110.3:c.596G>A NP_000101.2:p.Ser199Asn	rs776973423	Probably damaging (1)	Damaging (0.02)	No data/0/0.006371	.183
NM_000110.3:c.733A>T NP_000101.2:p.Ile245Phe	rs767836989	Possibly damaging (0.853)	Damaging (0)	No data/0/0.004376	.183
NM_000110.3:c.914C>A NP_000101.2:p.Thr305Lys	No number	Probably damaging (0.999)	Damaging (0.01)	No data/no data/no data	1
NM_000110.3:c.1003G>A NP_000101.2:p.Val335Met	rs72549306	Probably damaging (1)	Damaging (0)	0.12/0.01632/0.001989	1
NM_000110.3:c.1156G>T NP_000101.2:p.Glu386Ter	rs78060119			No data/0/0.0007974	.183
NM_000110.3:c.1627A>G NP_000101.2:p.Ile543Val	rs1801159	Benign (0)	Tolerated (0.44)	27.62/25.34/19.52	.974
NM_000110.3:c.1666A>C NP_000101.2:p.Ser556Arg	rs755407188	Probably damaging (1)	Damaging (0)	No data/0.02176/0.001596	1
NM_000110.3:c.1712C>A NP_000101.2:p.Ala571Asp	No number	Probably damaging (1)	Damaging (0)	No data/no data/no data	.183
NM_000110.3:c.1863G>T NP_000101.2:p.Trp621Cys	No number	Probably damaging (1)	Damaging (0)	No data/no data/no data	.183
NM_000110.3:c.2194G>A NP_000101.2:p.Val732Ile	rs1801160	Probably damaging (0.999)	Damaging (0)	19.7/1.887/4.531	.266
NM_000110.3:c.2303C>A NP_000101.2:p.Thr768Lys	rs56005131	Possibly damaging (0.579)	Damaging (0)	24.1/0.236/0.01948	.429
NM_000110.3:c.2476G>A NP_000101.2:p.Val826Met	No number	Probably damaging (0.975)	Damaging (0)	0.14/no data/no data	1
NM_000110.3:c.2678A>G NP_000101.2:p.Asn893Ser	rs188052243	Benign (0)	Tolerated (0.41)	0.22/0.04903/0.003989	1
DPYS					
NM_001385.2:c.-1T>C	rs2959023			69.14/70.45/59.17	.45
NM_001385.2:c.17G>A NP_001376.1:p.Arg6Gln	rs199618701	Benign (0.028)	Damaging (0.02)	0.13/0.3628/0.05538	1
NM_001385.2:c.541C>T NP_001376.1:p.Arg181Trp	rs36027551	Benign (0.024)	Tolerated (0.18)	3.02/5.928/0.9123	.0813
NM_001385.2:c.884A>G NP_001376.1:p.His295Arg	rs996605020	Probably damaging (0.985)	Tolerated (0.27)	No data/no data/no data	1
NM_001385.2:c.1001A>G NP_001376.1:p.Gln334Arg	rs121964923	Probably damaging (1)	Damaging (0)	0.41/0.06516/0.004597	.087
NM_001385.2:c.1253C>T NP_001376.1:p.Thr418Ile	No number	Probably damaging (1)	Damaging (0)	0.01/no data/no data	1
NM_001385.2:c.1469G>A NP_001376.1:p.Arg490His	rs189448963	Probably damaging (1)	Damaging (0)	0.06/0.01504/0.02369	1
UPB1					
NM_016327.2:c.91G>A NP_057411.1:p.Gly31Ser	rs200145797	Probably damaging (1)	Damaging (0)	0.12/0.4612/0.03339	1
NM_016327.2:c.977G>A NP_057411.1:p.Arg326Gln	rs118163237	Probably damaging (1)	Tolerated (0.29)	0.85/2.611/0.192	.671

A recent study has reported the functional characterization of 21 allelic variants of *DPYD* identified in 1070 Japanese individuals.[14] Five of the variants (p.Val335Met, p.Ile543Val, p.Val732Ile, p.Thr768Lys, p.Asn893Ser) identified in our present analysis were among those described in that earlier study. Among these 5 variants, the activity of the p.Val335Met and p.Thr768Lys mutant DPDs exhibited significantly lower intrinsic clearance (*CLint* = *Vmax*/*Km*) values compared to the wild-type enzyme (47.4% and 47.9% respectively). However, our present analysis did not find an association between any of these previously reported *DPYD* single variants and severe FP-related toxicity. This may be due to the small number of subjects we analyzed and a further investigation with an increased number of patients is thus warranted to further clarify this issue.

DHP is the second enzyme in the catabolic pathway of uracil and thymine. There are some reports of variants in *DPYS* that may explain the occurrence of severe toxicity from FP-based chemotherapy. For example, c.−1T>C is a common noncoding variant in this gene reported to have an impact on toxicity in patients receiving FP.[24] Our current results have also revealed a high allele frequency of 68% for c.−1T>C, but did not demonstrate a clear relationship between FP-related high toxicity and this variant. With regard to *DPYS* gene coding regions, a prior study has described a patient with severe adverse events from FP therapy harboring the *DPYS* compound heterozygous missense and nonsense variants p.Gly334Arg and p.Arg465Ter.[7] The p.Gln334Arg variant had been previously identified in Japanese patients with DHP deficiency and functional analysis revealed that the corresponding mutant enzyme had only 2.5% residual activity.[17] Until now, it was unknown whether a heterozygous p.Gly334Arg patient would be at a high risk for severe FP-related toxicity, but our current findings have suggested that this might be a possibility. Because the frequency of the p.Gly334Arg is higher in Japanese people than in other ethnic groups (0.41% vs 0.004597% Jmorp, genomeAD), genetic analysis of the *DPYS* gene is important, at least in Japanese patients. Conversely, our present data have indicated that no patients who are heterozygous for *DPYS* p.Arg181Trp developed a severe adverse event following FP treatment. The kinetic parameters of the corresponding mutant enzyme were assessed in a previous report and no markedly reduced activity relative to wild-type DHP was evident.[25] This variant may have protective effects against the development of FP-related toxicity in vivo, but the mechanism is unknown.

The contribution of the some *UPB1* gene alterations to the development of FP-related toxicity was also analyzed previously in White patients with cancer.[1] There have been few reports to date however on coding region variants in this gene. In our previous study, we revealed that the *UPB1* pathogenic variant c.977G>A p.Arg326Gln was prevalent in the Japanese population at a rate of 1.8% but was not found in more than 8000 European and more than 4000 African American alleles.[26] However, the association of this variant with FP-related toxicity is unknown. Our current results found no clear association between this *UPB1* variant and FP-related toxicity, suggesting that a standard regimen with this chemotherapeutic would be tolerated by heterozygous carriers of this pathogenic variant.

Rare *DPYD* variants that cause loss of function in silico and a *DPYS* pathogenic variant p.Gly334Arg may be associated with severe FP-related toxicity in Japanese patients with cancer. However, the common *UPB1* pathogenic variant p.Arg326Gln in the Japanese population does not show a clear association with toxicity in heterozygous individuals.

TABLE 4 Frequency of *DPYS* p.Arg181Trp, *DPYS* p.Gln334Arg and rare pathogenic *DPYD* variants found in the high-toxicity and low-toxicity groups

Genotype	Low toxicity (grade 0-2)	High toxicity (grade 3-4)	Total
DPYS c.541C>T (p.Arg181Trp)			
TT	0	0	0
CT	14	0	14
CC	232	55	287
Total	246	55	301
P-value = .0813			
DPYS c.1001A>G (p.Gln334Arg)			
GG	0	0	0
AG	1	2	3
AA	245	53	298
Total	246	55	301
P-value = .087			
Frequency of patients who had a rare and pathogenic variant of DPYD			
Hetero	2	5	7
Reference	244	50	294
Total	246	55	301
P-value = .0271			

TABLE 5 Clinical and genetic information for 2 heterozygous *DPYS* p.Gln334Arg patients with high toxicity

Patient no.	Age	Sex	Cancer	Regimen	Side effects	Other DPYD variants	Other DPYS variants	Other UPB1 variants
Patient 1	68	Female	Colorectal	5FU + L-OHP	Vomiting Grade 1 Neutropenia Grade 3	p.Ile543Val het p.Arg29Cys hom	c.-1T>C hom	No variant
Patient 2	81	Female	Colorectal	5FU + L-OHP	Nausea Grade 2 Neutropenia Grade 3	p.Arg29Cys het	c.-1T>C hom	No variant

TABLE 6 Detailed information on the 7 patients in the rare pathogenic *DPYD* variant group

Rare pathogenic variant	Age	Sex	Cancer	Regimen	Side effects	Other DPYD variants	Other DPYS variants	Other UPB1 variants
c.596G>A (p.Ser199Asn)	67	Female	Colorectal	5FU + L-OHP	Vomiting Grade 3 Diarrhea Grade 3 Neutropenia Grade 3	p.Ile543Val hom p.Arg29Cys hom	c.-1T>C het	No variant
c.733A>T (p.Ile245Phe)	63	Female	Stomach	5FU + L-OHP	Nausea Grade 2 Vomiting Grade 2 Diarrhea Grade 3	p.Ile543Val het p.Met166Val het p.Arg29Cys hom	c.-1T>C hom	No variant
c.914C>A (p.Thr305Lys)	66	Female	Colorectal	5FU + L-OHP	Diarrhea Grade 1 Oral Mucositis Grade 1 Hand-foot syndrome Grade 1 Neutropenia Grade 2	p.Arg29Cys hom	c.-1T>C het	No variant
c.1156G>T (p.Glu386Ter)	48	Male	Colorectal	FOLFOXIRI + α	Nausea Grade 1 Neutropenia Grade 3	p.Arg29Cys hom	c.-1T>C hom	No variant
c.1666A>C (p.Ser556Arg)	25	Male	Colorectal	5FU + L-OHP	Nausea Grade 1 Vomiting Grade 1 Oral Mucositis Grade 1 Neutropenia Grade 1	p.Arg29Cys hom	c.-1T>C hom	No variant
c.1712C>A (p.Ala571Asp)	70	Female	Colorectal	5FU mono	Nausea Grade 3 Diarrhea Grade 3 Neutropenia Grade 1	p.Arg29Cys het	c.-1T>C hom	No variant
c.1863G>T (p.Trp621Cys)	72	Female	Colorectal	5FU + L-OHP	Nausea Grade 1 Neutropenia Grade 3	p.Ile543Val het p.Arg29Cys hom p.Thr768Lys het	c.-1T>C hom	No variant

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ETHICAL APPROVAL

We obtained approval for this study from the Ethical Review Board for Human Genome Studies at Fujita Health University. Written informed consent was obtained from all patients. All experiments were carried out in accordance with the relevant guidelines and regulations.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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