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

Katsuyuki Yokoi1,2 | Yoko Nakajima1 | Hiroshi Matsuoka3 | Yasuko Shinkai2 | Takuma Ishihara4 | Yasuhiro Maeda5 | Takema Kato2 | Hidetoshi Katsuno3 | Koji Masumori3 | Kenji Kawada6 | Tetsushi Yoshikawa1 | Tetsuya Ito1 | Hiroki Kurahashi2

1Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Japan
2Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Japan
3Department of Gastrointestinal Surgery, Fujita Health University School of Medicine, Toyoake, Japan
4Innovative and Clinical Research Promotion Center, Gifu University Hospital Gifu University, Gifu, Japan
5Center for Joint Research Facilities Support, Fujita Health University, Toyoake, Japan
6Department of Medical Oncology, Fujita Health University School of Medicine, Toyoake, Japan

Correspondence
Yoko Nakajima, Department of Pediatrics, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi 470-1192, Japan.
Email: yonaka@fujita-hu.ac.jp

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; jUP, β-ureidopropionase.

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1 | INTRODUCTION

Fluoropyrimidines including 5FU and its prodrugs are widely used in the treatment of various malignancies including head and neck, gastrointestinal, or breast cancers. 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. 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. Patients with a DPD deficiency have an increased risk of developing severe treatment-related toxicity from a standard dose of FP. 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. 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, DPYD13, I560S) and c.1236G>A (rs56038477, E412E, in haplotype B3). Hence, DPYD genotype-guided dose individualization of FP therapy has now been conducted in some western countries. 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. Recently, 21 DPYD allelic variants were identified in 1070 healthy Japanese individuals. The functional alterations caused by these variants were analyzed in vitro and their enzyme activities were characterized. 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. The DHP-encoding gene, DPYS, is located on chromosome 8q22 and the βUP-encoding gene, UPB1, is located on chromosome 22q11. A relationship between DPYS and UPB1 gene variations and severe FP-related toxicity has been reported, 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). We have also reported that some DPYS variants may be more common than expected in East Asian groups. 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 > 50x. Allele frequency was investigated with gnomAD browser beta (http://gnomad.broadinsti tute.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/phil2) 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 0.05 was considered statistically significant, and $0.05 < P < 0.1$ 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 (81.7%)              | 55 (18.3%)               |
| Age      |              |                          |                          |
| Median   | 67           | 66 (86.6%)              | 68 (13.4%)              |
| 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.
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 is 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 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.

### Discussion

More than 450 DPYD variants have been identified to date as a cause of 5FU-related toxicity in patients with cancer. 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. However, none of these DPYD variants has been identified to date in an Asian population. 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. 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.

| 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%)                           |

| Table 2: Numbers and proportions (%) of patients experiencing different categories of toxicity during the first 2 therapy cycles |
| 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    | rs1801265     | Benign (0)                      | Tolerated (0.18)         | 96.85/92.8/76.6                                 | .507    |
| NP_000101.2:p.Arg29Cys |               |                                 |                          |                                                 |         |
| NM_000110.3:c.496A>G   | rs2297595     | Probably damaging (1)           | Tolerated (0.07)         | 2.18/1.524/8.585                                | .146    |
| NP_000101.2:p.Met166Val|               |                                 |                          |                                                 |         |
| NM_000110.3:c.596G>A   | rs776973423   | Probably damaging (1)           | Damaging (0.02)          | No data/0/0.006371                               | .183    |
| NP_000101.2:p.Ser199Asn|               |                                 |                          |                                                 |         |
| NM_000110.3:c.733A>T   | rs76836989    | Possibly damaging (0.853)       | Damaging (0)             | No data/0.004376                                 | .183    |
| NP_000101.2:p.Ile245Phe|               |                                 |                          |                                                 |         |
| NM_000110.3:c.914C>A   | rs72549306    | Probably damaging (1)           | Damaging (0)             | 0.12/0.01632/0.001989                            | 1       |
| NP_000101.2:p.Val335Met|               |                                 |                          |                                                 |         |
| NM_000110.3:c.1003G>A  | rs78060119    | No number                       | Damaging (0)             | No data/0.007974                                 | .183    |
| NP_000101.2:p.Val335Met|               |                                 |                          |                                                 |         |
| NM_000110.3:c.1156G>T  | rs1801159     | Benign (0)                      | Tolerated (0.44)         | 27.62/25.34/19.52                               | .974    |
| NP_000101.2:p.Glu386Ter|               |                                 |                          |                                                 |         |
| NM_000110.3:c.1627A>G  | rs1801160     | Probably damaging (0.999)       | Damaging (0)             | No data/no data/no data                          |         |
| NP_000101.2:p.Ile543Val|               |                                 |                          |                                                 |         |
| NM_000110.3:c.1666A>C  | rs755407188   | Probably damaging (1)           | Damaging (0)             | No data/0.02176/0.001596                          | 1       |
| NP_000101.2:p.Val732Ile|               |                                 |                          |                                                 |         |
| NM_000110.3:c.1712C>A  | No number     | Probably damaging (1)           | Damaging (0)             | No data/no data/no data                          | .183    |
| NP_000101.2:p.Ala571Asp|               |                                 |                          |                                                 |         |
| NM_000110.3:c.1863C>T  | No number     | Probably damaging (1)           | Damaging (0)             | No data/no data/no data                          | .183    |
| NP_000101.2:p.Trp621Cys|               |                                 |                          |                                                 |         |
| NM_000110.3:c.2194G>A  | rs1801160     | Probably damaging (0.999)       | Damaging (0)             | 19.7/1.887/4.531                                | .266    |
| NP_000101.2:p.Val732Ile|               |                                 |                          |                                                 |         |
| NM_000110.3:c.2303C>A  | rs56005131    | Possibly damaging (0.579)       | Damaging (0)             | 24.1/0.236/0.01948                               | .429    |
| NP_000101.2:p.Thr768Lys|               |                                 |                          |                                                 |         |
| NM_000110.3:c.2476G>A  | No number     | Probably damaging (0.975)       | Damaging (0)             | 0.14/no data/no data                             | 1       |
| NP_000101.2:p.Val826Met|               |                                 |                          |                                                 |         |
| NM_000110.3:c.2678A>G  | rs188052243   | Benign (0)                      | Tolerated (0.41)         | 0.22/0.04903/0.003989                            | 1       |
| NP_000101.2:p.Asni8935er|               |                                 |                          |                                                 |         |
| **DPYS**               |               |                                 |                          |                                                 |         |
| NM_001385.2:c.-1T>C    | rs2959023     | Benign (0.028)                  | Damaging (0.02)          | 0.13/0.3628/0.05538                              | 1       |
| NP_001376.1:p.Arg6Gln  |               |                                 |                          |                                                 |         |
| NM_001385.2:c.541C>T   | rs36027551    | Benign (0.024)                  | Tolerated (0.18)         | 3.02/5.928/0.9123                                | .0813   |
| NP_001376.1:p.Arg181Trp |               |                                 |                          |                                                 |         |
| NM_001385.2:c.884A>G   | rs996605020   | Probably damaging (0.985)       | Tolerated (0.27)         | No data/no data/no data                          | 1       |
| NP_001376.1:p.His295Arg|               |                                 |                          |                                                 |         |
| NM_001385.2:c.1001A>G  | rs121964923   | Probably damaging (1)           | Damaging (0)             | 0.41/0.06516/0.004597                            | .087    |
| NP_001376.1:p.Gln334Arg|               |                                 |                          |                                                 |         |
| NM_001385.2:c.1253C>T  | No number     | Probably damaging (1)           | Damaging (0)             | 0.01/no data/no data                             | 1       |
| NP_001376.1:p.Gln595Trp|               |                                 |                          |                                                 |         |
| NM_001385.2:c.1469G>A  | rs189448963   | Probably damaging (1)           | Damaging (0)             | 0.06/0.01504/0.02369                             | 1       |
| NP_001376.1:p.Arg940His |               |                                 |                          |                                                 |         |
| **UPB1**               |               |                                 |                          |                                                 |         |
| NM_016327.2:c.91G>A    | rs200145797   | Probably damaging (1)           | Damaging (0)             | 0.12/0.4612/0.03339                              | 1       |
| NP_057411.1:p.Gly315Ser|               |                                 |                          |                                                 |         |
| NM_016327.2:c.977G>A   | rs118163237   | Probably damaging (1)           | Tolerated (0.29)         | 0.85/2.611/0.192                                 | .671    |
| NP_057411.1:p.Arg326Gln|               |                                 |                          |                                                 |         |
A recent study has reported the functional characterization of 21 allelic variants of DPYD identified in 1070 Japanese individuals. Five of the variants (p.Val335Met, p.Ile543Val, p.Val732Ile, p.Thr768Lys, p.Asn893Ser) identified in our present analysis were among those described in 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.

DPYD is the second enzyme in the catabolic pathway of uracil and thymine. There are some reports of variants in this gene in Japanese patients with cancer 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. 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. 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. 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 0.00497% Jmorps, 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. 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. 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. 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 | p.Ile543Val het | p.Arg181Trp | c.-1T>C hom | No variant |
| Patient 2   | 81  | Female | Colorectal | 5FU + L-OHP | Nausea Grade 2 | p.Arg29Cys hom | p.Arg181Trp | 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 | p.Ile543Val hom | c.-1T>C het | No variant |
| c.733A>T (p.Ile245Phe)  | 63  | Female | Stomach | 5FU + L-OHP | Nausea Grade 2 | p.Ile543Val het | c.-1T>C hom | No variant |
| c.914C>A (p.Thr305Lys)  | 66  | Female | Colorectal | 5FU + L-OHP | Diarrhea Grade 1 | p.Arg29Cys hom | c.-1T>C het | No variant |
| c.1156G>T (p.Glu386Ter) | 48  | Male | Colorectal | FOLFOXIRI + α | Nausea Grade 1 | p.Arg29Cys hom | c.-1T>C hom | No variant |
| c.1666A>C (p.Ser556Arg) | 25  | Male | Colorectal | 5FU + L-OHP | Nausea Grade 1 | p.Arg29Cys hom | c.-1T>C hom | No variant |
| c.1712C>A (p.Ala571Asp) | 70  | Female | Colorectal | 5FU mono | Nausea Grade 3 | p.Arg29Cys het | c.-1T>C hom | No variant |
| c.1863G>T (p.Trp621Cys) | 72  | Female | Colorectal | 5FU + L-OHP | Nausea Grade 1 | p.Ile543Val 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.

ORCID
Katsuyuki Yokoi https://orcid.org/0000-0002-4760-0420

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

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