


BRIEF COMMUNICATION

Poor association between dihydropyrimidine dehydrogenase (*DPYD*) genotype and fluoropyrimidine-induced toxicity in an Asian population

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Abstract

Objective: Dihydropyrimidine dehydrogenase (*DPYD*) genotype is closely associated with fluoropyrimidine (FP)-induced toxicities in Caucasian population and European Medicines Agency now recommends *DPYD* genotype-based FP dosing strategy.

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Patients and Methods: The current study aimed to investigate their impact on FP-related toxicities in an Asian population using genome-wide association study (GWAS) data set from 1364 patients with colon cancer.

Results: Among 82 variants registered in the Clinical Pharmacogenetics Implementation Consortium, 74 *DPYD* variants were directly genotyped in GWAS cohort; however, only 7 nonsynonymous *DPYD* variants (CPIC variants) were identified and none of the four recurrent *DPYD* variants (*DPYD**2A, c.2846A>T, c.1679T>G, c.1236G>A) were included. Seven CPIC variants were investigated for their association with the incidence of FP-related toxicities; however, none of these variants revealed a significant correlation with FP-related toxicities.

Conclusion: These data suggested that the *DPYD* genotype registered in CPIC plays a minor role in FP-related toxicities in an Asian population.

KEYWORDS

Asian population, *DPYD*, fluoropyrimidine, genotyping, toxicity

1 | INTRODUCTION

Fluoropyrimidine (FP) is widely used for a variety of cancers; however, some patients suffer from severe toxicities such as diarrhea, mucositis, hand-foot syndrome, and neutropenia. Dihydropyrimidine dehydrogenase (*DPYD*) is a key enzyme in FP degradation and approximately 8% of the Caucasian population have reduced *DPYD* genotype and 0.1% having no *DPYD* enzyme activity.^{1,2} Since impaired *DPYD* variants delay FP degradation, patients harboring these variants have the increased risk of developing severe FP-related toxicities. A recent prospective study demonstrated that FP dose adjustment based on the four recurrent *DPYD* variants (*DPYD**2A, c.2846A>T, c.1679T>G, c.1236G>A) could reduce the risk of FP-related toxicities in the Caucasian population.² A cost analysis revealed that upfront *DPYD* genotyping did not increase the burden of medical expenses and that expected total costs per patient were comparable regardless of whether or not they underwent universal screening.³ Based on the aforementioned data, the European Medicines Agency (EMA) has recommended universal *DPYD* genotyping before initiating FP-based chemotherapy in 2020⁴ and Clinical Pharmacogenetics Implementation Consortium (CPIC) has published 82 *DPYD* variants including 27 variants with impaired enzyme activity⁵; however, it remains unclear whether universal *DPYD* screening and genotype-based FP dose adjustment are feasible for the Asian population. Therefore, the current study aimed to investigate the prevalence of *DPYD* variants registered in CPIC and its association with FP-related toxicities using GWAS data

from 1364 patients with colon cancer who underwent adjuvant chemotherapy under clinical trials.

2 | METHODS

2.1 | Genotyping and clinical data obtained from a genome-wide association study targeting colon cancer who underwent adjuvant chemotherapy

Genotyping and clinical data obtained from a genome-wide association study (GWAS) targeting the 1364 patients with colon cancer who underwent adjuvant chemotherapy under clinical trials (JOIN or ACIEVE) were utilized. Among 82 *DPYD* variants registered in the CPIC⁵ (CPIC variants), 74 variants were directly genotyped in GWAS cohort. JOIN was a phase II trial to test the safety of 6 months of modified FOLFOX6 (mFOLFOX6) in Japanese patients with colon cancer.⁶ In contrast, ACHIEVE was a phase III trial planned as a part of phase III trials of the International Duration Evaluation of Adjuvant Chemotherapy (IDEA),⁷ and tested 3 versus 6 months of mFOLFOX6 or capecitabine plus oxaliplatin (CAPOX) adjuvant chemotherapy in patients with colon cancer.^{8,9} Diarrhea/mucositis/hand-foot syndrome/neutropenia were selected as representative FP-related toxicities and were graded according to the CTCAE version 3.0 (JOIN) and 4.0 (ACHIEVE), respectively. Given those essential elements of diarrhea, mucositis, hand-foot syndrome, and neutropenia of CTCAE versions 3.0 were equivalent to those of version 4.0, data from both clinical

trials were comparable. In both clinical trials, clinical data were collected using an electronic data capture system with regular central monitoring. The primary outcomes of the GWAS were published elsewhere.¹⁰

2.2 | Comprehensive search for *DPYD* variants using the Japanese Haplotype Reference Panel database

Nonsynonymous variants of *DPYD* were comprehensively searched using the registry database of the Japanese Haplotype Reference Panel (JHRP, $n = 3135$. <https://www.hgvd.genome.med.kyoto-u.ac.jp/>).

2.3 | Statistical analysis

Associations between the presence of FP-related toxicities during adjuvant chemotherapy and genotype frequencies of nonsynonymous *DPYD* variants were investigated. p values and odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using logistic regression analysis according to the additive genetic model. The significance level was set according to Bonferroni's correction for multiple testing with nine variants ($p < 0.005$). All statistical analyses

were performed using R (version 3.2.0; <http://www.r-project.org/>).

2.4 | Ethics

The protocols of GWAS study were approved by the institutional review boards of all participating institutions, and all patients provided written informed consent for the use of genomic and clinical data for research purposes.

3 | RESULTS

3.1 | Prevalence of *DPYD* variants registered in CPIC guidelines in GWAS cohort

Among 82 *DPYD* variants registered in CPIC, 74 variants were directly genotyped in GWAS cohort; however, only seven nonsynonymous variants (CPIC variants) were identified and none of the four most clinically relevant *DPYD* variants (*DPYD**2A, c.2846A>T, c.1679T>G, c.1236G>A) in the Caucasian population were included. Furthermore, these seven CPIC variants were presumed to have normal function according to the CPIC guidelines (Table 1).

TABLE 1 Seven CPIC and two non-CPIC *DPYD* variants were found in the GWAS cohort

rs number	Nucleotide change/ amino acid substitution	CPIC/non-CPIC	Presumed <i>DPYD</i> activity	Minor allele frequency	
				GWAS cohort ($n = 1364$)	JHRP cohort ($n = 3135$)
rs56005131	c.C2303A p.T768K	CPIC	Normal	0.022	0.025
rs1801160	c.G2194A p.V732I	CPIC	Normal	0.019	0.018
rs1801159	c.A1627G p.I543V	CPIC	Normal	0.28	0.27
rs142512579	c.G1294A p.D432N	CPIC	Normal	0.00036	0
rs72549306	c.G1003T p.V335L	CPIC	Normal	0.00073	0
rs2297595	c.A496G p.M166V	CPIC	Normal	0.02	0.019
rs200562975	c.A451G p.N151D	CPIC	Normal	0.0029	0.0013
rs188052243	c.A2678G p.N893S	Non-CPIC	Decreased	0.0011	0.0022
rs1801265	c.C85T p.R29C	Non-CPIC	Unknown	0.04	0.04

TABLE 2 Association between the presence of each nonsynonymous *DPYD* variants and fluoropyrimidine-related toxicities in the GWAS cohort ($n = 1364$)

rs number	Toxicities	Minor allele frequency in patients with indicated toxicity (%)	Odds ratio	95% Confidence interval	p-value
rs56005131	Diarrhea	4.1	1.8	0.62–5.2	0.26
G>T	Mucositis	1.7	0.79	0.18–3.4	0.74
	Neutropenia	2.2	0.99	0.50–1.9	0.98
	Hand-foot syndrome	1.3	0.36	0.049–2.7	0.49
rs1801160	Diarrhea	4.1	2.2	0.75–6.4	0.14
C>T	Mucositis	0	N/A	N/A	N/A
	Neutropenia	1.8	0.92	0.44–1.8	0.82
	Hand-foot syndrome	2.7	1.6	0.16–14.6	0.36
rs1801159	Diarrhea	25.5	0.84	0.53–1.3	0.46
T>C	Mucositis	38.3	1.5	1.0–2.2	0.024
	Neutropenia	29.4	1.0	0.84–1.2	0.7
	Hand-foot syndrome	28.0	0.98	0.49–1.9	0.92
rs142512579	Diarrhea	0	N/A	N/A	N/A
C>T	Mucositis	0	N/A	N/A	N/A
	Neutropenia	0	N/A	N/A	N/A
	Hand-foot syndrome	0	N/A	N/A	N/A
rs72549306	Diarrhea	1	21.1	1.2–341.5	0.032
C>A	Mucositis	0.89	19.4	1.2–315.7	0.036
	Neutropenia	0.15	N/A	N/A	N/A
	Hand-foot syndrome	0	N/A	N/A	N/A
rs2297595	Diarrhea	5.1	3.4	1.2–9.0	0.015
T>C	Mucositis	2.6	1.3	0.40–4.5	0.61
	Neutropenia	2.2	1.1	0.56–2.2	0.71
	Hand-foot syndrome	2.7	1.3	0.20–9.3	0.59
rs200562975	Diarrhea	0	N/A	N/A	N/A
T>C	Mucositis	0	N/A	N/A	N/A
	Neutropenia	0.47	5.1	0.53–49.4	0.15
	Hand-foot syndrome	0	N/A	N/A	N/A
rs188052243	Diarrhea	0	N/A	N/A	N/A
T>C	Mucositis	0	N/A	N/A	N/A
	Neutropenia	0	N/A	N/A	N/A
	Hand-foot syndrome	0	N/A	N/A	N/A
rs1801265	Diarrhea	5.1	1.6	0.64–4.4	0.3
A>G	Mucositis	2.3	1.19	0.25–2.7	0.76
	Neutropenia	2.0	0.53	0.28–1.0	0.054
	Hand-foot syndrome	1.3	0.4	0.049–2.7	0.21

Note: As for diarrhea and neutropenia, patients who developed grade 3 or 4 events were counted. As for mucositis and hand-foot syndrome, patients who developed grade 2 or higher events were counted. “N/A” meant that odds ratio could not be estimated due to having zero cases. Uncorrected p -values were shown.

3.2 | Comprehensive search for *DPYD* variants using JHRP database

Comprehensive search for *DPYD* variants using the JHRP database identified 53 exonic (1 frameshift

insertion, 39 nonsynonymous, and 13 synonymous) variants (Table S1). Eleven variants were overlapped with those registered in CPIC guidelines. However, the four recurrent *DPYD* variants were not registered in the JHRP database comprising 3135 Japanese subjects. Among the

42 variants not registered in CPIC guidelines, two variants were directly genotyped in GWAS cohort and were identified (non-CPIC variants). One variant (rs188052243) had been reported to have decreased enzymatic activity in vitro (Table 1).¹¹

3.3 | Association between seven CPIC and two non-CPIC DPYD variants and FP-related toxicities

The prevalence of the seven CPIC and two non-CPIC DPYD variants were compared between those who had grade 0 and grade ≥ 2 or 3 diarrhea, mucositis, hand-foot syndrome, and neutropenia; however, no significant associations were observed (Table 2).

4 | DISCUSSION

Since 2020, EMA has recommended universal DPYD genotyping before initiating FP-based chemotherapy.⁴ In this study, we aimed to investigate the feasibility of DPYD genotype-based FP dosing strategy in an Asian population. The four most clinically relevant DPYD variants (DPYD*2A, c.2846A>T, c.1679T>G, c.1236G>A) in the Caucasian population were not found in both GWAS cohort ($n = 1364$) and the JHRP database ($n = 3135$), which were in line with the previous study searching for DPYD variants in 341 Japanese subjects.¹² Comprehensive search for nonsynonymous DPYD variants using the JHRP database identified 42 DPYD variants which were not published in the CPIC guideline; however, most of them were rare and median minor allele frequency (MAF) was 0.0006 (range 0.0001–0.28, Table S1). Only two variants were directly genotyped and were identified in GWAS cohort. Notably, no significant association had been found between seven CPIC and two non-CPIC DPYD variants identified in the GWAS cohort and FP-related toxicities. Two patients harboring presumed impaired DPYD enzymatic activity (rs188052243) did not develop severe FP-related toxicities.

Thus, the prevalence of DPYD genotype registered in CPIC guideline is quite low and plays a minor role in FP-related toxicities in an Asian population. In contrast, incidence of FP-related toxicities in Japanese patients were comparable or higher than those in Caucasian patients (Table S2). For example, the incidence of grade 3 or higher neutropenia observed in this cohort (23.2%) was significantly higher compared to that in the IDEA trial excluding ACHIEVE cohort (8.2%) (Table S2). This suggests that the development of FP-related toxicities

in the Asian population could be attributed to factors other than the DPYD variants registered in CPIC guideline.

We also evaluated the association between other hematological toxicities (leucopenia, anemia, thrombocytopenia, and febrile neutropenia) and nine non-synonymous DPYD variants in our GWAS cohort; however, no significant associations were found (data not shown).

The current study has some limitations. First, although data regarding FP-related toxicities were collected in large prospective clinical trials, their actual relationship with FP was unclear given that patients received a combination chemotherapy of FP and oxaliplatin. Second, most of rare DPYD variants identified in Japanese subjects with presumed impaired enzymatic activity^{11,13} were not directly genotyped in GWAS cohort. Therefore, we could not exclude the possibility of rare DPYD variants were involved in FP-related toxicities in the Asian population.

In summary, the current study suggested that DPYD genotypes registered in CPIC guidelines play a minor role in FP-related toxicities in the Asian population and that universal DPYD screening does not appear to be feasible.

AUTHOR CONTRIBUTIONS

Masashi Kanai: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (equal); project administration (lead); writing – original draft (lead). **Takahisa Kawaguchi:** Data curation (lead); formal analysis (lead); methodology (lead); validation (lead); writing – original draft (lead). **Masahito Kotaka:** Investigation (equal). **Dai Manaka:** Investigation (equal). **Junichi Hasegawa:** Investigation (equal). **Akinori Takagane:** Investigation (equal). **Yoshinori Munemoto:** Investigation (equal). **Takeshi Kato:** Investigation (equal). **Tetsuya Eto:** Investigation (equal). **Tetsuo Touyama:** Investigation (equal). **Takanori Matsui:** Investigation (equal). **Shinozaki Katsunori:** Investigation (equal). **Shigemi Matsumoto:** Conceptualization (supporting); investigation (equal). **Tsunekazu Mizushima:** Investigation (equal). **Masaki Mori:** Project administration (supporting). **Junichi Sakamoto:** Project administration (supporting); writing – review and editing (lead). **Atsushi Ohtsu:** Project administration (supporting). **Takayuki Yoshino:** Conceptualization (lead); project administration (lead); supervision (lead). **shigetoyo saji:** Project administration (lead); supervision (lead). **Fumihiko Matsuda:** Conceptualization (lead); project administration (lead); supervision (lead); writing – review and editing (equal).

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CONFLICT OF INTEREST

Masashi Kanai reports stock from Therabiopharma Inc., honoraria from Chugai Pharmaceutical Co., Ltd., and research grant from Molecular Health. Masahito Kotaka reports honoraria from Chugai Pharmaceutical Co., Ltd., and Yakult Honsha Co., Ltd. Takeshi Kato reports honoraria from Chugai Pharmaceutical Co., Ltd., Takeda Pharmaceutical Company Limited, Taiho Pharmaceutical Co., Ltd, ONO Pharmaceutical Co., and Eli Lilly Japan K.K. Katsunori Shinozaki reports honoraria from Chugai Pharmaceutical Co., Ltd., Mochida Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co., Ltd., Eli Lilly Japan K.K., Daiichi Sankyo Co. Ltd., Bayer Yakuhin, Ltd., Ono Pharmaceutical Co., Ltd., Eisai Co., Ltd., SHIONOGI & Co., Ltd., Merck Biopharma Co., Ltd., Nippon Kayaku Co., Ltd., and Kyowa Kirin Co., Ltd. Takayuki Yoshino reports honoraria from Taiho Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Eli Lilly Japan K.K., Merck Biopharma Co., Ltd., Bayer Yakuhin, Ltd., Ono Pharmaceutical Co., and MSD K.K., and research funds from MSD K.K. (Inst), Daiichi Sankyo Co., Ltd. (Inst), Ono Pharmaceutical Co., Ltd (Inst), Taiho Pharmaceutical Co., Ltd (Inst), Amgen K.K. (Inst), Sanofi K.K. (Inst), Pfizer Japan Inc, (Inst), Genomedica (Inst), Sysmex Corporation (Inst), Nippon Boehringer Ingelheim Co., Ltd (Inst), PAREXEL International Inc. (Inst), Chugai Pharma Co., Ltd (Inst). Fumihiko Matsuda reports research grant from Yakult Honsha Co., Ltd.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The protocols of GWAS study were approved by the institutional review boards of all participating institutions, and all patients provided written informed

consent for the use of genomic and clinical data for research purposes.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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