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POLYMORPHISM OF *DIHYDROPYRIMIDINE DEHYDROGENASE (DPYD)* Cys29Arg AND RISK OF SIX MALIGNANCIES IN JAPANESE

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ABSTRACT

Dihydropyrimidine dehydrogenase (DPD) is the enzyme catalyzing the first step of pyrimidine metabolism. To date, genetic polymorphisms of pyrimidine-synthesizing enzymes have been reported to be associated with the risk of malignant lymphoma or colon cancer. Accordingly, there may be associations between *dihydropyrimidine dehydrogenase (DPYD)* polymorphism and the risk of malignancies. We conducted a prevalent case-control study to investigate the associations between a functional polymorphism of dihydropyrimidine dehydrogenase, *DPYD* T85C, and the risk of six malignancies. Controls were 445 Nagoya City inhabitants without a history of malignancy who had participated in a health check-up between August and September 2000. Case subjects were 901 patients with malignancies (99 esophageal, 131 gastric, 143 colon, 179 lung, 243 breast, and 106 malignant lymphomas) who had visited Aichi Cancer Center Hospital between March 1999 and December 2000. No *DPYD CC* individuals were found in either cases or controls. The frequency of *DPYD TC* genotype was 6.3% in control subjects and 5.9% in all case subjects (not significant). In a subgroup analysis, the frequency of *TC* genotype was highest in patients with gastric cancer (9.1%), followed by those with lung cancer (8.3%), with the lowest frequency in those with malignant lymphoma (1.9%). The gender- and age- adjusted odds ratios and 95% confidence intervals for the *TC* genotype of gastric cancer and malignant lymphoma were 1.52 (0.71–3.28) and 0.31 (0.71–1.34), respectively. Although prevalent cases were used, this study suggested that the influence of *DPYD* T85C posed only a limited risk for the six malignancies.

Key Words: Dihydropyrimidine dehydrogenase, Genetic polymorphism, Cancer risk

INTRODUCTION

Dihydropyrimidine dehydrogenase (DPD) acts as the first-step enzyme catabolizing pyrimidine-like thymine or uracil *in vivo* (Fig 1). Although DPD activity is widely seen in various organs, its activity is especially high in hepatic cells or peripheral blood mononuclear cells.[1)]

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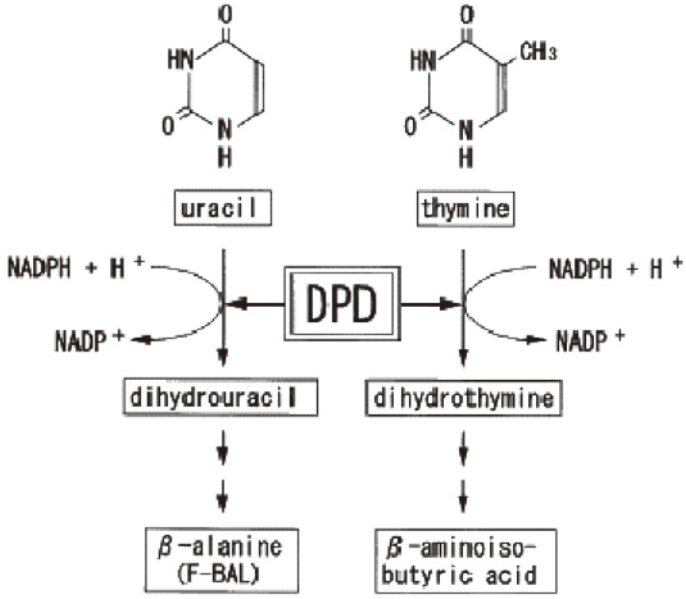

Fig. 1 Metabolic pathways of pyrimidines and dihydropyrimidine dehydrogenase (DPD).

DPD also metabolizes 5-FU, one of the most widely used anti-cancer drugs, more than 80% of which is catalyzed by this enzyme.[2] The adverse reactions caused by this drug differ from one individual to another, which is supposedly attributable to the differences in DPD activity.

To date, a congenital deficiency of this enzyme has been found, which is clinically called dihydropyrimidine dehydrogenase deficiency (DPD deficiency).[3,4] The etiology of this disease is the accumulation of large amounts of pyrimidine caused by a congenital lack of DPD activity. There are no consistent symptoms in this disease, and most of the patients were encountered by chance. Although epilepsy is the most frequent symptom, mental retardations, microcephaly,[5-7] and neurological symptoms have also been reported, though their association with pyrimidine metabolism disorder has not yet been definitively clarified.[8,9] This disease is suspected when abnormally high levels of thymine or uracil in blood or urine samples are detected by HPLC (High Performance Liquid Chromatography) or gas chromatography. In addition, loss of activity in the measurement of DPD enzyme activity using live liver tissues, cultured fibroblasts, or peripheral mononuclear cells also helps to diagnose this disease, although unreliability has been reported in these methods.[10] The first case of this disorder was reported in 1984 in the Netherlands.[11] In Japan, 3 cancer patients who presented severe adverse reactions from 5-FU treatment were reported to be diagnosed as DPD deficiency.[12] In a screening survey of pyrimidine metabolism disorder in our country using 34,200 urine samples of infants, 3 children with pyrimidine metabolism disorder without any clinical manifestation were found.[13]

Thirty-nine polymorphic sites have been revealed in the *dihydropyrimidine dehydrogenase (DPYD)* gene to date,[14] including one splice-site mutation, 5 frame-shift mutations, 2 nonsense mutations, 8 intron-site mutations, and 23 single nucleotide polymorphisms. Among them, 31 gene mutations were detected in DPD deficiency patients;[15-18] a splice-site mutation IVS14+1G/A, two nonsense mutations, R21X and E386X, which changed the conformation of this enzyme, and D949V and V335L, which prevented cofactor bindings and electron conduction, all of which are

possible candidates for the cause of DPD deficiency.[18,19] T85C (Cys29Arg) is located in exon 2, in which the *C* allele reportedly produces DPD of lower enzyme activity.[20,21] On the other hand, one report has demonstrated sufficient DPD enzyme activity in human peripheral blood mononuclear cells,[22] which renders the influence of *DPYD* genotype on its enzyme activity somewhat controversial.

To date, polymorphisms in genes involved in the synthesis of thymine or uracil have been reported to be associated with the risk of human cancers. For example, genetic polymorphisms of thymidylate synthase (TS) and methylenetetrahydrofolate reductase (MTHFR) have both been demonstrated to modulate the risk of malignant lymphoma[23-26] and colon cancer.[27-29] It seems biologically plausible to hypothesize an association between the gene polymorphisms in *DPYD*, which catalyze pyrimidine bases, and susceptibility to cancers, though no reports on such association have yet been published. Accordingly, in this study, we investigated the associations between *DPYD* T85C polymorphism with a relatively high frequency of a minor allele[30] and the risk of six malignancies using a prevalent case-control population.

MATERIALS AND METHODS

Study population and sample collection

Case subjects were 901 voluntary patients with malignancies (99 esophageal, 131 gastric, 143 colon, 179 lung, 243 breast, and 106 malignant lymphomas) who had visited the Aichi Cancer Center Hospital between March 1999 and December 2000.[31] Control samples were solicited from 489 inhabitants of Nagoya City, who had participated in a health checkup between August and September 2000. Those with a history of malignancy were excluded.[32] Subjects who gave written informed consent for participation in this study were asked to provide blood from a peripheral vein. All patients and control subjects were Japanese. This study was approved by the Institutional Review Board of the Aichi Cancer Center (approved numbers 12–20 (breast cancer), 12–13 (esophageal cancer), 12–27 (other malignancies), and 12–23 (control subjects)).

Genotyping analysis of DPYD Cys29Arg polymorphism

DNA of each subject was extracted from the buffy-coat fraction with a QIAamp DNA Blood Mini Kit (Quiagen, Valencia, CA).

Genotypes were determined by polymerase chain reaction with confronting two-pair primers (PCR-CTPP).[33] The primers used were as follows: F1: 5'-AGG TTG CAG TGA ACT GAG ATT GTA-3', R1: 5'-CCT GGC CGA AGT GGA AC<u>A</u>-3' for the *T* allele, and F2: 5'-ACA CAA ACT CAT GCA ACT CTG <u>C</u>-3', R2: 5'-TTG CCT TAC AAT GTG TGG AGT G-3' for the *C* allele. Those underlined are the bases of the single nucleotide polymorphism. Genomic DNA (30 ng to 100 ng) was used per 25 μl of reaction with 0.2 mM dNTPs, 12.5 pmol of each primer, 0.75 units of "AmpliTaq Gold," and 2.5 μl GeneAmp 10×PCR Buffer, including 15 mM $MgCl_2$ (Perkin-Elmer Corp., Foster City, CA). Amplification conditions were 10 minutes of initial denaturation at 95°C, followed by 30 cycles of 1 minute at 95°C, 1 minute at 67°C, and 1 minute at 72°C, followed by a 5-minute final extension at 72°C. The amplified DNA was visualized on a 2% agarose gel with ethidium bromide staining. A representative gel is shown in Fig 3. Genotyping of *DPYD* T85C polymorphism was distinguished as follows: a 150-bp band for the *C* allele, a 219-bp band for the *T* allele, as well as a 330-bp common band.

Statistical analysis

All statistical analyses in this study were performed using STATA (College Station, TX)

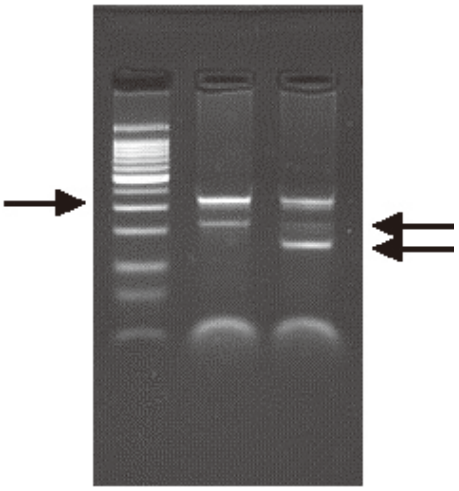

Fig. 2 Polymorphism of the *DPYD* T85C (Cys29Arg)
Left lane denotes a 100-bp DNA ladder, center lane, *TT* genotype (330- and 219-bp bands); right lane, *TC* genotype (330-, 219-, and 150-bp bands).
Each band is distinguished as follows: a 150-bp band for the *C* allele, a 219-bp band for the *T* allele, as well as a 330-bp common band.

statistical software. Accordance with the Hardy-Weinberg equilibrium, which indicates the absence of a discrepancy between genotype and allele frequencies, was checked in control subjects using the χ^2 test. Odds ratios (ORs) and 95% confidence intervals (95% CI) were adjusted for sex and age as continuous variables using an unconditional logistic regression model. Adjustment for multiple comparisons was not performed, since the analyses were conducted in an exploratory context, which requires careful interpretation of any P values.

RESULTS

Table 1 shows the genotype frequencies, age- and sex- adjusted odds ratios (OR), and 95% confidence intervals (95%CI) for *DPYD* T85C polymorphism. No *DYPD CC* individual was found in either cases or controls. The frequencies of *DPYD TT, TC, and CC* genotypes in control subjects were 93.7%, 6.3% and 0.0%, respectively. The genotype frequency of *DPYD* T85C polymorphism was in accordance with the Hardy-Weinberg equilibrium (χ^2 = 0.470, p = 0.4932).

The frequency of *TC* genotype was 5.9% in all case subjects, and no statistically significant difference from that of the controls was observed. In the subgroup analysis, the frequencies of *TC* genotype were 4.0% for esophageal, 9.2% for gastric, 6.3% for colon, 5.0% for lung, 5.0% for breast, and 1.9% for malignant lymphoma patients.

As shown in Table 1, the gender- and age- adjusted OR for *TC* genotype of all cancers was 0.78 (95% CI: 0.47–1.29). For each cancer, the ORs for *TC* genotype were 0.65 (0.20–2.09) for esophageal, 1.52 (0.71–3.28) for gastric, 1.00 (0.45–2.24) for colon, 0.73 (0.32–1.63) for lung, 0.58 (0.25–1.35) for breast, and 0.31 (0.71–1.34) for malignant lymphoma patients, none of which were statistically significant.

Table 1 Sex- and age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of six malignancies for *DPYD* T85C (Cys29Arg) polymorphism

Cases/Controls	Age	n	Sex male/female	*DPYD* T85C polymorphism (%) *TT*	*TC*	*CC*
Controls	32-85	445	126/319	417 (93.7)	28 (6.3)	0 (0)
Cases						
All malignancies	20–84	901	423/478	850 (94.3)	51 (5.7)	0 (0)
OR (95%CI)				1	0.78 (0.47–1.29)	—
Esophageal	43–84	99	84/15	95 (96.0)	4 (4.0)	0 (0)
OR (95%CI)				1	0.65 (0.20–2.09)	—
Gastric	32–82	131	91/40	119 (90.8)	12 (9.2)	0 (0)
OR (95%CI)				1	1.52 (0.71–3.28)	—
Colon	27–82	143	80/63	134 (93.7)	9 (6.3)	0 (0)
OR (95%CI)				1	1.00 (0.45–2.24)	—
Breast	20–72	243	234 (96.3)	9 (4.7)	0 (0)	
OR (95%CI)				1	0.58 (0.25–1.35)	—
Lung	31–84	179	109/70	164 (91.6)	15 (8.4)	0 (0)
OR (95%CI)				1	0.73 (0.32–1.63)	—
Malignant lymphoma	20–83	106	59/47	104 (98.1)	2 (1.9)	0 (0)
OR (95%CI)				1	0.31 (0.71–1.34)	—

% in parentheses.

DISCUSSION

In the present case-control study using 901 cases with malignancies, we observed no statistically significant association between *DYPD* T85C polymorphism and susceptibility to the six malignancies. However, we found a tendency for those with *DPYD TC* genotype to be at a higher risk of gastric cancer, but a lower risk of malignant lymphoma. As already mentioned, malignant lymphoma risk has been demonstrated to be modulated by polymorphisms in the thymine-synthesizing enzymes, in several reports[23-26] while there are few reports ever concerning thymine-synthesizing enzymes and gastric cancer risk.[34]

The genotype frequency of *DYPD* T85C polymorphism in Japanese has been reported in about 104 cancer patients and 3 healthy individuals in Saitama Prefecture,[30] revealing that the *C*-allele frequency was 3.7%, a value similar to that in our study (3.1%). A study of 300 Taiwanese subjects has revealed that a *C*-allele frequency of 4.3%, and no *CC* genotype was observed.[35] However, a German Study of 157 Caucasians demonstrated a higher value of 19.4%,[36] indicating that the *DPYD* 85 *C* allele is more frequently observed in Caucasians than in Orientals.

DPYD T85C polymorphism, in which cysteine without electron polarity at 29th amino acid is substituted for arginine with electron polarity, might reveal a difference in its enzyme activity. Some gene-expression experiments using *E. coli* showed that the enzyme activity of DPD encoded by the 85 *C* allele was too low to be detected.[20,21] In a study of 37 cancer patients, DPD activity was measured by the HPLC method after inducing reactions by adding substrates and enzymes to the DPD extracted from peripheral blood mononuclear cells.[16] Genotyping of 14 cases who presented with more than 30% lower activities than those in healthy individuals disclosed 4 cases with *DPYD* T85C polymorphisms, all of whom were *TC* genotype. Among them, one case with

the lowest enzyme activity demonstrated a 92% loss of activity, while the remaining three cases showed losses of 47% to 69%. The actual influence of *DPYD* T85C polymorphism in the real environment, i.e., in cells or live human organs, remains to be clarified.

Another study of 37 cancer patients was also reported.[22] In this study, the *C*-allele frequency was 33.8%. Among these case subjects, 2 had *CC* genotype. When DPD enzyme activities were measured by the HPLC method using peripheral blood mononuclear cells of those two cases, one showed a loss of activity, while the other showed normal activity. Considering that individuals with *CC* genotype do not lose their enzyme activities, and that *C* alleles are highly prevalent in cancer patients, the abovementioned study supported their hypothesis that *DPYD* T85C polymorphism might not exert any remarkable influence on DPD functions. To verify this hypothesis, further *in vivo* studies will be required, which may well prove controversial, given the possibility that various factors other than *DPYD* T85C genotypes may also be involved.

In conclusion, this study suggested that the influence of *DPYD* T85C were limited for the risk for six malignancies among Japanese. Since this is a prevalent case-control study, and the prognosis of the case subjects could affect the results, further studies of incident case-control populations will be required to resolve this question.[37] Studies of this association among other ethnic groups also remain to be done.

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