Identification of Novel Mutations in the Dihydropyrimidine Dehydrogenase Gene in a Japanese Patient with 5-Fluorouracil Toxicity[1]

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ABSTRACT

5-Fluorouracil (5-FU) is used widely in the treatment of several common neoplasms. Dihydropyrimidine dehydrogenase (DPD) is the initial and rate-limiting enzyme in the catabolism of 5-FU. Several recent studies have described a pharmacogenetic disorder in which cancer patients with decreased DPD activity develop life-threatening toxicity following exposure to 5-FU. We reported recently the first Japanese case of decreased DPD activity accompanied by severe 5-FU toxicity. The present study describes the results of molecular analysis of this patient and her family, in which three novel mutations (Arg21Gln, Val335Leu, and Glu386Ter) of the gene coding for DPD were identified. We also revealed that Arg21Gln and Glu386Ter are on the same allele and that Val335Leu is on the other allele, on the basis of analysis of the family genome. Expression analysis in *Escherichia coli* showed that Val335Leu and Glu386Ter led to mutant DPD protein with significant loss of enzymatic activity and no activity, respectively. The Arg21Gln mutation, however, resulted in no decrease in enzymatic activity compared with the wild type. The present data represent the first molecular genetic analysis of DPD deficiency accompanied by severe 5-FU toxicity in a Japanese patient.

INTRODUCTION

In humans, catabolism of uracil and thymine takes place through a three-step pathway leading to the generation of β-alanine and β-aminoisobutyric acid (1). DPD[3] (EC 1.3.1.2) is the initial and rate-limiting enzyme of the pathway (2).

DPD is also the key enzyme that degrades the structurally related pyrimidine antimetabolite 5-FU and its analogues. These chemotherapeutic drugs are used widely in the management of several common malignancies, including cancer of the gastrointestinal tract, ovary, and breast (3). In humans, >85% of administered 5-FU is degraded through this catabolic pathway (4).

DPD deficiency (McKusick 274270) was described first in pediatric patients exhibiting thymine-uraciluria associated with a variety of neurological symptoms (5–8). Family studies have shown that the DPD deficiency follows an autosomal recessive pattern of inheritance, based on measurements of catalytic activity in lymphocytes (9, 10). On the other hand, DPD deficiency that demonstrates no clinical symptoms has also been reported (11, 12).

Several recent studies have described a pharmacogenetic disorder in which individuals with decreased DPD activity develop life-threatening toxicity following exposure to 5-FU (9, 13–16).

The recent cloning of cDNA and genomic DNA encoding DPD has allowed detection of the defect at the molecular level (17, 18). These studies have demonstrated exon skipping, deletion, and some missense mutations in Caucasian patients (10–12, 19, 20).

In Japan, until recently there have been no reports of DPD deficiency. We first reported a Japanese female with decreased DPD activity accompanied by severe 5-FU toxicity in 1997 (21). The present study describes the results of molecular analysis of this patient and her family.

MATERIALS AND METHODS

Patient. The proband was a 57-year-old woman who was diagnosed with breast cancer. She presented with leukopenia, thrombocytopenia, and severe mucositis during 5-FU therapy, and the level of DPD activity in her peripheral blood mononuclear cells was decreased significantly (21).

Pyrimidine Metabolite Concentration in Urine. Urinary pyrimidines and their derivatives, such as uracil, dihydrouracil, thymine, and dihydrothymine, were measured as reported previously (22).

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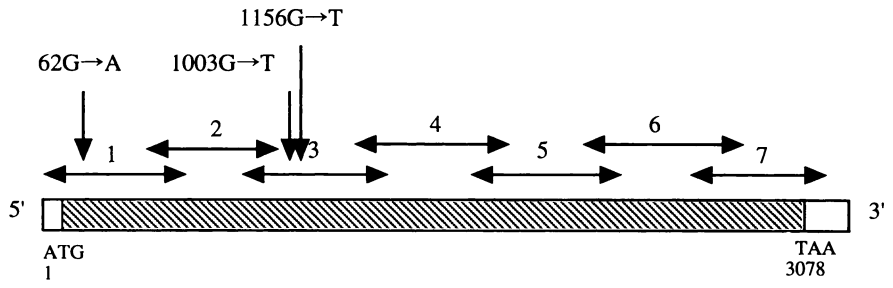
[3] The abbreviations used are: DPD, dihydropyrimidine dehydrogenase; 5-FU, 5-fluorouracil; RT, reverse transcription; Ter, termination codon.

a



b

Amplicon	Nucleotide positions	Sense primer(5'-3')	Antisense primer (5'-3')
position 1	-46 - 450	GCAAGGAGGGTTTGTCACTG	AATGGGTCCCTCTTCAGTGG
2	370 - 877	CTGACTTGTGGAATGGTATG	GGCATCTTTATTGGGTTCTG
3	831 - 1362	AGCTGCTTTCATTGGAATAG	TATAGGGCTCAAGGCTTCTT
4	1307 - 1774	GTGCCTTTGGTTCAGTTCTG	GGATGATTCTGGGGGAAACA
5	1711 - 2224	GCCCTCACCAAAACTTTCTC	TCAGACCTGAGACAGTGTTG
6	2162 - 2659	CAAGAGCTGCAAAGGAAGGT	TGCGCTGTTCCAGATAAGGT
7	2615 - 3132	TGGACAAGAAACTGCCAAGT	GATCAGCATATGTAGGTGAC

c

	length(bp)	sense primer(5'-3')	antisense primer (5'-3')
exon 2	126	TTTAGAGTATCCTGGCTTTA	TGGTACTTACAAAGCAGTTC
exon 10	171	GTGCGCCTGTCACTCTCCAT	TTTACCTCCTCAGGGACAGC
exon 11	199	TAGATGGAACTTGCTAAGGA	GAACTGAACCAAAGGCACTG

Fig. 1 *a*, diagram of human DPD cDNA. *Striped portion* corresponds to coding region. *Open boxes* represent 5′ and 3′ noncoding regions. Initiation *ATG* and stop *TAA* codons are indicated. *Horizontal arrows* represent the seven regions amplified by RT-PCR. *Vertical arrows* indicate the site of each mutation. *b*, sequence of oligonucleotide primers used for PCR amplification of cDNA fragments containing whole coding sequence. Nucleotide position of each PCR product is indicated. *c*, sequence of oligonucleotide primers used for PCR amplification of genomic fragments containing DPD exons. Predicted length of each PCR product is indicated.

DPD Catalytic Activity. DPD catalytic activity in the proband and her family members was determined in peripheral blood mononuclear cells using a previously described method (Ref. 23; Kyowa Analytical Research Center, Shizuoka, Japan).

Isolation of Total RNA and RT-PCR. Whole blood was obtained from the proband. Total RNA was extracted from peripheral blood leukocytes using ISOGEN (Nippon Gene, Tokyo), a procedure based on acid guanidine thiocyanate-phenol-chloroform extraction (24). The RNA solutions were dissolved in diethyl pyrocarbonate-treated water and stored at −80°C until use. RT was carried out as described (10), using 5 μg of total RNA. The coding sequence (17) was divided into seven parts, and PCR primers were designed to amplify each part so that the 3′ end portion of each amplified fragment could overlap the 5′ end portion of the next fragment (Fig. 1*a*). The sequences of the primers are shown in Fig. 1*b*. An aliquot of the cDNA was amplified by each PCR. PCR was carried out in 50 μl of reaction mixture consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM dNTPs, 1 μM each primer, and 2.5 units of Taq polymerase (Perkin-Elmer Cetus, San Jose, CA) for 30 cycles of denaturing at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The products were analyzed by electrophoresis on a 1.0% agarose gel and visualized by staining with ethidium bromide.

Mutation Detection. To identify mutations, RT-PCR products were subcloned into the pCR 2.1 vector (Invitrogen Corp., San Diego, CA) and subjected to cDNA sequence analysis. The cDNA clones were sequenced on a 373A automated DNA sequencer (Applied Biosystems, Foster City, CA), using the PRISM dye-terminator cycle sequence kit (Applied Biosystems). At least eight independent clones were sequenced for each part of the cDNA clone (Fig. 1*a*). Each sequence was determined from both strands.

Genomic DNA Analysis. To identify the family genotype and confirm the sequence alterations, we performed genomic DNA analysis by PCR amplification, using peripheral blood leukocyte genomic DNA from the proband as well as other available members of the family (her mother, sister 1, and sister 2). Genomic DNA was prepared from whole blood by the standard methods (25). Each exon (exons 2, 10, and 11) of the gene coding for DPD was amplified using exon-specific primer pairs (Fig. 1*c*) (18). In exons 2 and 11, RFLP analysis was performed to demonstrate the predicted site alterations in their amplified fragments. The PCR product was digested to completion with the appropriate restriction enzymes and analyzed by electrophoresis on a 3.0% agarose gel. In exon 10, the PCR product was subcloned and sequenced to screen for mutations.

Construction of Expression Plasmids. Expression plasmids were constructed in the vector pSE420 (Invitrogen) using the human DPD cDNA. First, part of the polylinker sequence in pSE420 between the *Sal*I and *Hin*dIII sites was deleted by cleavage with these enzymes and religation. The resulting vector pSE420ΔSH was cut with *Nco*I and *Eco*RI, and the DPD *Nco*I-*Eco*RI fragment, spanning the entire coding region and part of the 3′ untranslated region up to the *Bsp*HI site, was ligated, resulting in the plasmid pSE420-DPD. [The vector pSE420ΔSH and the plasmid pSE420-DPD were kindly supplied by Dr. P. Vreken (26)]. PCR-based mutagenesis by a two-stage method (27), using the pSE420-DPD as template, was used to create mutations Arg21Gln, Val335Leu, and Glu386Ter. Each recom-

Table 1 Pyrimidine metabolite concentrations in urine samples of patient and controls

	URA[a] (μmol/g creatinine)	DHU (μmol/g creatinine)	DHT/URA	THY (μmol/g creatinine)	DHT (μmol/g creatinine)	DHT/THY
Proband	141.7	17.3	0.12	17.3	2.12	0.29
Control	66.3 ± 30.0	17.5 ± 5.3	0.31 ± 0.16	1.2 ± 0.6	3.8 ± 1.8	3.7 ± 1.7

[a] URA, uracil; DHU, dihydrouracil; THY, thymine; DHT, dihydrothymine.

Table 2 Characteristic mutations detected in the gene coding for DPD

Family member	DPD activity in lymphocyte[a]		Mutation	Effect of mutation
Proband	11	(allele 1)	1003G → T	Val335Leu
		(allele 2)	62G → A &	Arg21Gln
			1156G → T	Glu386Ter[b]
Mother	55	(allele 1)	1003G → T	Val335Leu
		(allele 2)	wt[c]	
Sister 1	41	(allele 1)	1003G → T	Val335Leu
		(allele 2)	wt	
Sister 2	ND	(allele 1)	1003G → T	Val335Leu
		(allele 2)	62G → A &	Arg21Gln
			1156G → T	Glu386Ter

[a] DPD activity is expressed as pmol/min/mg protein. DPD activity in normal control was 258.2 ± 144.8 pmol/min/mg protein (adult, n = 10).
[b] Ter, termination codon leading to a truncated peptide of 385 amino acids.
[c] wt, wild type; ND, not detectable (<2 pmol/min/mg protein).

binant mutant clone was sequenced to verify the presence of the mutation and the absence of any other alterations. The resulting clones were designated pSE420-DPD-Arg21Gln, pSE420-DPD-Val335Leu and pSE420-DPD-Glu386Ter, respectively. The 893-bp *Nco*I-*Eco*NI-digested fragment from the pSE420-DPD-Arg21Gln was ligated into *Nco*I-*Eco*NI -digested pSE420-DPD-Glu386Ter plasmid, resulting in pSE420-DPD-Arg21Gln/Glu386Ter.

Expression Analysis of Missense Mutation in *Escherichia coli*. Expression plasmids were introduced into *E. coli* strain BL21(F-,*ompT*, $hsdS_b(r_bm_b)$, gal, dcm). A 20-ml Luria-Bertani broth culture, supplemented with 100 μg/ml ampicillin, 100 μM uracil, 100 μM each of flavin adenine dinucleotide and flavin mononucleotide, and 10 mM each of Na_2S and $Fe(NH_4)_2(SO_4)$ was inoculated with 100 μl of an overnight preculture grown in Luria-Bertani broth. Cells were grown in the culture medium to an absorbance at 550 nm of 0.5–0.6 at 30°C, and induction was performed by the addition of 1 mM isopropyl-1-thio-galactopyranoside. Cells were precipitated by centrifugation at a 2-h time point, washed with isolation buffer [35 mM potassium phosphate (pH 7.3), 10 mM EDTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, 2 μM leupeptin, and 2 μM pepstatin], and resuspended in the same buffer. The cell suspension was frozen at −20°C for at least 16 h, thawed on ice, and lysed by sonication. The crude lysate was centrifuged at 20,000 × g for 15 min. The resulting supernatant was used for the DPD activity assay (26). The assay was performed by the same method described previously (Ref. 23; Otsuka Assay Laboratory, Tokushima, Japan).

The level of exogenous DPD enzymatic activity was determined by subtracting the DPD enzymatic activity of pSE420ΔSH from that of each expression plasmid.

RESULTS

Pyrimidine Metabolite Concentration in Urine. As shown in Table 1, urinary thymine and uracil concentrations were increased in the proband.

DPD Catalytic Activity. DPD catalytic activity of the proband and her family ranged from an undetectable level to 55 pmol/min/mg protein. The DPD catalytic activity of the normal control in this study was 258.2 ± 144.8 pmol/min/mg protein (adult, n = 10). All of the family members presented with relatively low DPD catalytic activity, <25% of the mean in the normal control (Table 2).

cDNA Sequence Analysis. We analyzed the cDNA sequences containing the whole coding region of the proband and found three nucleotide changes (Table 2, Fig. 2). First, there was a G→A transition in exon 2 (position 62 on the cDNA, 62G→A), which caused a missense mutation (Arg21Gln). Second, in exon 10, there was a G→T transversion in exon 10 (1003G→T, Val335Leu). Third, there was a G→T transversion in exon 11, which caused premature termination at codon 386. This leads to a truncated peptide of 385 amino acids (stop codon; 1156G→T, Glu386Ter). These three mutations were detected based on four of eight, five of eight, and three of eight clones analyzed, respectively, indicating a heterozygotic state.

Mutation Analysis of Genomic DNA. Two point mutations (62G→A and 1156G→T) were expected to disrupt restriction endonuclease sites in the corresponding exons, permitting the confirmation of their presence and segregation analysis by RFLP (Fig. 3). With regard to the mutation in exon 10 (1003G→T), the PCR product was subcloned and sequenced to screen for mutations.

Family screening revealed that sister 2 had the same DPD genotype as the proband. The mother and sister 1 did not demonstrate the two mutations in exons 2 and 11 (Arg21Gln and

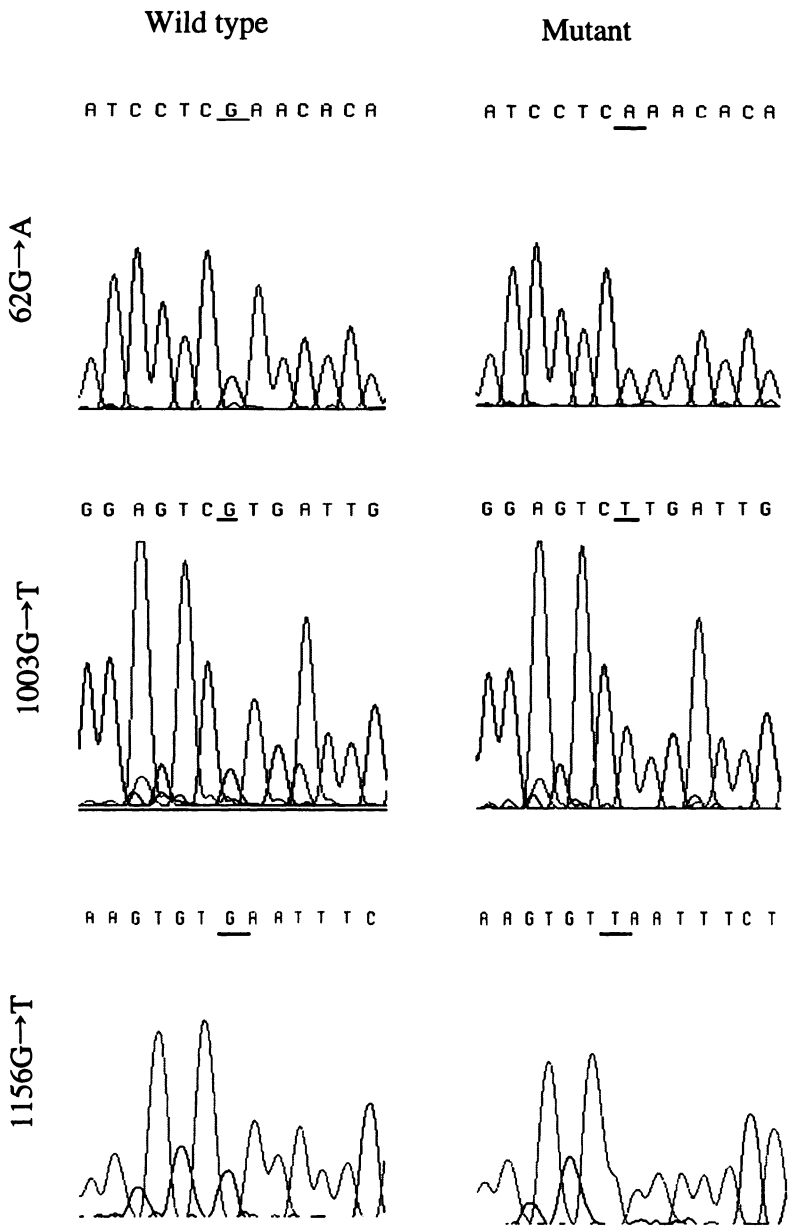


Fig. 2 Detection of mutations in DPD alleles of proband and family members. For each mutation, genomic sequence changes are shown in automated tracings for region surrounding mutation site (*underlined*) from both wild-type controls and mutant alleles.

Glu386Ter). However, they both demonstrated the mutation in exon 10 (Val335Leu). These data show that the two mutations in exons 2 and 11 (Arg21Gln and Glu386Ter) are on the same allele and that the mutation in exon 10 (Val335Leu) is on the other allele (Table 2, Fig. 3).

Expression of Mutant Proteins. To examine the functional defects of mutant proteins, three mutant genes were introduced into the pSE420-DPD (wild-type) expression vector. The endogenous DPD enzymatic activity in the *E. coli* strain used for expression of the constructs was below the limit of detection of the assay (26). The construct containing wild-type DPD cDNA expressed DPD activity approximately 13-fold above the background activity of pSE420ΔSH vector. The exogenous DPD enzymatic activity, from which the background activity was subtracted, is summarized in Table 3. The pSE420-DPD-Val335Leu construct shows significant loss of the activity, and the constructs of pSE420-DPD-Glu386Ter and pSE420-DPD-Arg21Gln/Glu386Ter constructs show no activity; the construct of pSE420-DPD-Arg21Gln shows no decrease in enzymatic activity (Table 3).

DISCUSSION

We identified three novel mutations of the gene that codes for DPD in a Japanese family with decreased DPD activity. The proband was a compound heterozygote who presented with a 62G→A mutation in exon 2 (Arg21Gln) and a 1156G→T mutation in exon 11 (Glu386Ter) on one allele,

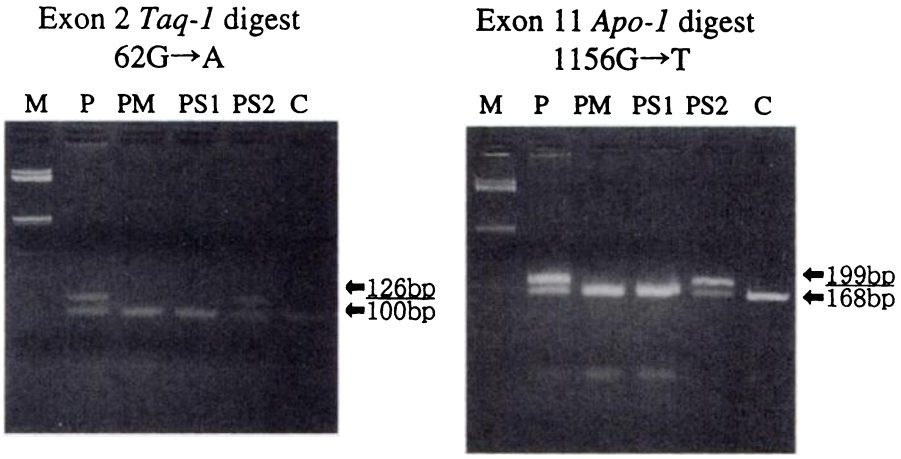


Fig. 3 RFLP analysis of the point mutation. PCR-amplified exons from proband (*P*), her mother (*PM*), her sisters (*PS1* and *PS2*), and unrelated individuals (*C*) were digested with restriction enzymes (*Taq*1, *Apo*1). Molecular weight standard (*M*) loaded on left lane of each gel is Col EI *Hae*III digest. Length of restriction fragments corresponding to restriction site changes resulting from mutations are *underlined*. In *Taq*1 digestion of exon 2 fragment (*Taq-1*), wild-type allele yields 100- and 26-bp fragments, whereas heterozygotes containing 62G→A reveal both these bands and full-length 126-bp band. In *Apo*1 digestion of the exon 11 fragment (*Apo-1*), wild-type allele yields 168- and 31-bp fragments, whereas heterozygotes containing 1156G→T reveal both these bands and full-length 199-bp band.

Table 3 Enzymatic activity of mutant DPD proteins

Construct	DPD enzymatic activity[a] (mean ± 1 SD, n = 4)
pSE420-DPD (wild type)	209.0 ± 127.2
pSE420-DPD-Arg21Gln	202.5 ± 60.2
pSE420-DPD-Val335Leu	35.7 ± 35.0
pSE420-DPD-Glu386Ter	ND[b]
pSE420-DPD-Arg21Gln/Glu386Ter	ND

[a] DPD enzymatic activity represents the relative exogenous DPD activity measured in *E. coli* lysates (pmol/min/mg protein).
[b] ND, not detectable.

and a 1003G→T mutation in exon 10 (Val335Leu) on the other allele. Sister 2 had the same DPD genotype as the proband, whereas the mother and sister 1 had only the missense mutation in exon 10.

The mutation in exon 11 causes a premature termination (1156G→T, Glu386Ter). Because the premature stop codon is located before the putative uracil-binding site encoded by nucleotides 1960–1993 (17), this Glu386Ter mutant protein has no residual activity. In fact, using expression analysis, we revealed that this mutant DPD protein did not express any residual activity (Table 3).

On the other hand, the mutation in exon 10 (1003G→T, Val335Leu) was located at the putative NADPH binding-site encoded by nucleotides 1001–1053 (17). Using expression analysis, we revealed that this mutant protein showed about 15% residual DPD activity (Table 3). Moreover, the DPD catalytic activity in the peripheral mononuclear cells of the mother and sister 1 was <25% of the activity of a normal control (Table 2). Therefore, we concluded that Val335Leu is responsible for the reduction of DPD catalytic activity. Although the mother and sister 1 were heterozygotes, they presented with extremely low DPD catalytic activity in the peripheral blood mononuclear cells (Table 2). This apparent discrepancy between the genotype and DPD catalytic activity may be explained by the fact that DPD exists as a homodimer in humans (17, 28). If two intact subunits are required for DPD enzymatic activity, a heterozygous individual would be expected to express only 25% of the activity of a normal control.

With regard to the mutation in exon 2 (62G→A, Arg21Gln), expression analysis of the pSE420-DPD-Arg21Gln construct showed no decrease in enzymatic activity compared with the pSE420-DPD. This result indicates that Arg21Gln is unlikely to be responsible for the catalytic activity of DPD (Table 3).

Until now several mutations of the gene that codes for DPD have been described, including exon skipping and some missense mutations (10–12, 19–20). Skipping of exon 14 (165 bp), which is caused by a G→A point mutation in an invariant GT splice donor sequence, appears to be a common mutation. A study of Caucasian populations revealed an incidence of 3–5% in the heterozygous state of this mutation. On the basis of these results, up to 1 in every 1000 births in the general population might be homozygous for DPD mutation (20). However, this G→A mutation had not been identified previously in the Japanese population (70 alleles).

Severe 5-FU toxicity was reported recently in members of a Caucasian population with heterozygous DPD deficiency (20). In the present study, we identified three novel mutations in a Japanese patient with severe 5-FU toxicity. 5-FU and its analogues are the most commonly prescribed chemotherapeutic drugs for cancer treatment in Japan. This suggests that 5-FU toxicity in DPD deficiency is a serious problem not only among Caucasians but also among Japanese.

Additional studies, such as population study to determine the frequency of these genetic defects, are presently under investigation in our laboratory. We believe that this study is the first step toward preventing 5-FU toxicity in DPD deficiency in Japan.

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REFERENCES

1. Wasternack, C. Degradation of pyrimidines and pyrimidine analogs–pathways and mutual influences. Pharmacol. & Ther., *8:* 629–651, 1980.

2. Gonzalez, F. J., and Fernandez-Salguero, P. Diagnostic analysis, clinical importance and molecular basis of dihydropyrimidine dehydrogenase deficiency. Trends Pharmacol. Sci., *16:* 325–327, 1995.

3. Chabner, B. A., and Myers, C. E. Clinical pharmacology of cancer chemotherapy. *In:* V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Cancer, Principles and Practice of Oncology. pp.287–328. Philadelphia, PA: J. B. Lippincott Co., 1985.

4. Heggie, G. D., Sommadossi, J. P., Cross, D. S., Huster, W. J., and Diasio, R. B. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. Cancer Res., *47:* 2203–2206, 1987.

5. Bakkeren, J. A., De Abreu, R. A., Sengers, R. C., Gabreels, F. J., Maas, J. M., and Renier, W. O. Elevated urine, blood and cerebrospinal fluid levels of uracil and thymine in a child with dihydrothymine dehydrogenase deficiency. Clin. Chim. Acta, *140:* 247–256, 1984.

6. Berger, R., Stoker-de Vries, S. A., Wadman, S. K., Duran, M., Beemer, F. A., de Bree, P. K., Weits-Binnerts, J. J., Penders, T. J., and van der Woude, J. K. Dihydropyrimidine dehydrogenase deficiency leading to thymine-uraciluria. An inborn error of pyrimidine metabolism. Clin. Chim. Acta, *141:* 227–234, 1984.

7. Wadman, S. K., Berger, R., Duran, M., de Bree, P. K., Stoker-de Vries, S. A., Beemer, F. A., Weits-Binnerts, J. J., Penders, T. J., and van der Woude, J. K. Dihydropyrimidine dehydrogenase deficiency leading to thymine-uraciluria. An inborn error of pyrimidine metabolism. J. Inherit. Metab. Dis., *8:* 113–114, 1985.

8. Brockstedt, M., Jakobs, C., Smit, L. M., van Gennip, A. H., and Berger, R. A new case of dihydropyrimidine dehydrogenase deficiency. J. Inherit. Metab. Dis., *13:* 121–124, 1990.

9. Diasio, R. B., Beavers, T. L., and Carpenter, J. T. Familial deficiency of dihydropyrimidine dehydrogenase. Biochemical basis for familial pyrimidinemia and severe 5-fluorouracil-induced toxicity. J. Clin. Invest., *81:* 47–51, 1988.

10. Meinsma, R., Fernandez-Salguero, P., Van Kuilenburg, A. B., Van Gennip, A. H., and Gonzalez, F. J. Human polymorphism in drug metabolism: mutation in the dihydropyrimidine dehydrogenase gene results in exon skipping and thymine uraciluria. DNA Cell Biol., *14:* 1–6, 1995.

11. Vreken, P., Van Kuilenburg, A. B., Meinsma, R., De Abreu, R. A., and Van Gennip, A. H. Identification of a four-base deletion (delTCAT296–299) in the dihydropyrimidine dehydrogenase gene with variable clinical expression. Hum. Genet., *100:* 263–265, 1997.

12. Fernandez-Salguero, P. M., Sapone, A., Wei, X., Holt, J. R., Jones, S., Idle, J. R., and Gonzalez, F. J. Lack of correlation between phenotype and genotype for the polymorphically expressed dihydropyrimidine dehydrogenase in a family of Pakistani origin. Pharmacogenetics, *7:* 161–163, 1997.

13. Diasio, R. B., and Lu, Z. Dihydropyrimidine dehydrogenase activity and fluorouracil chemotherapy. J. Clin. Oncol., *12:* 2239–2242, 1994.

14. Tuchman, M., Stoeckeler, J. S., Kiang, D. T., O'Dea, R. F., Ramnaraine, M. L., and Mirkin, B. L. Familial pyrimidinemia and pyrimidinuria associated with severe fluorouracil toxicity. N. Engl. J. Med., *313:* 245–249, 1985.

15. Harris, B. E., Carpenter, J. T., and Diasio, R. B. Severe 5-fluorouracil toxicity secondary to dihydropyrimidine dehydrogenase deficiency. A potentially more common pharmacogenetic syndrome. Cancer (Phila.), *68:* 499–501, 1991.

16. Houyau, P., Gay, C., Chatelut, E., Canal, P., Roche, H., and Milano, G. Severe fluorouracil toxicity in a patient with dihydropyrimidine dehydrogenase deficiency. J. Natl. Cancer Inst., *85:* 1602–1603, 1993.

17. Yokota, H., Fernandez-Salguero, P., Furuya, H., Lin, K., McBride, O. W., Podschun, B., Schbackerz, K. D., and Gonzalez, F. J. cDNA cloning and chromosome mapping of human dihydropyrimidine dehydrogenase, an enzyme associated with 5-fluorouracil toxicity and congenital thymine uraciluria. J. Biol. Chem., *269:* 23192–23196, 1994.

18. Johnson, M. R., Wang, K., Tillmanns, S., Albin, N., and Diasio, R. B. Structural organization of the human *dihydropyrimidine dehydrogenase* gene. Cancer Res., *57:* 1660–1663, 1997.

19. Vreken, P., Van Kuilenburg, A. B., Meinsma, R., Beemer, F. A., Duran, M., and Van Gennip, A. H. Dihydropyrimidine dehydrogenase deficiency: a novel mutation and expression of missense mutation in *E. coli*. J. Inherit. Metab. Dis., *21:* 276–279, 1998.

20. Wei, X., McLeod, H. L., McMurrough, J., Gonzalez, F. J., and Fernandez-Salguero, P. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. J. Clin. invest., *98:* 610–615, 1996.

21. Ohe, I., Dobashi, K., Miyazaki, M., and Mori, H. The first Japanese case of severe 5-fluorouracil toxicity secondary to dihydropyrimidine dehydrogenase. J. Jpn. Soc. Cancer Ther., *35:* 796, 1997.

22. Sumi, S., Kidouchi, K., Ohba, S., and Wada, Y. Automated screening system for purine pyrimidine metabolism disorders using high-performance liquid chromatography. J. Chromatogr. B. Biomed. Appl., *672:* 233–239, 1995.

23. Harris, B. E., Song, R., Soong S. J., and Diasio, R. B., Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. Cancer Res., *50:* 197–201, 1990.

24. Chomczynski, P. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques, *15:* 532–534, 1993.

25. Maniatis, T., Fritsch, E. F., and Sambrook, J. Isolation of high-molecular-weight DNA from mammalian cells. *In:* T. Maniatis, E. F. Fritsch, and J. Sambrook (eds.), Molecular Cloning, A Laboratory Manual, 2nd ed., pp. 9.17–9.19. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.

26. Vreken, P., Van Kuilenburg, A. B. P., Meinsma, R., and Van Gennip, A. H. Dihydropyrimidine dehydrogenase (DPD) deficiency: identification and expression of missense mutations C29R, R886H, and R235W. Hum. Genet., *101:* 333–338, 1997.

27. Higuchi, R., Drummel, B., and Saiki, R. K. A general method of *in vitro* preparation and specific mutagenesis of DNA fragments: study of protein and DNA interactions. Nucleic Acids Res., *16:* 7351–7367, 1988.

28. Takai, S., and Fernandez-Salguero, P., Kimura, S., Gonzalez, F. J., and Yamada, K. Assignment of the human dihydropyrimidine dehydrogenase gene (*DYPD*) to chromosome region 1p22 by fluorescence in situ hybridization. Genomics, *24:* 613–614, 1994.

Clinical Cancer Research

Identification of novel mutations in the dihydropyrimidine dehydrogenase gene in a Japanese patient with 5-fluorouracil toxicity.

M Kouwaki, N Hamajima, S Sumi, et al.

Clin Cancer Res 1998;4:2999-3004.