a) Block 1

Nucleotide change			-609C>T	-471T>G	-216C>A	-213G>A	39C>A	85T>C	IVS2+153 T>C	IVS3+33 A>G	325T>A	451A>G	474T>C	IVS5-115G>A	496A>G	639C>T	733A>T	IVS7+64 G>T	793G>A	IVS8+91 C>T	IVS9-128A>T	1003G>T	IVS10+24 A>G	IVS10-15T>C	Number	Frequency
Amino acid change							A13E	C29R			Y109N	N151D	F158F		M166V	D213D	I245F		E265K			V335L				
Haplotype	*1	*1a																							558	0.818
		*1b																							31	0.045
		*1c																							14	0.021
		*1d																							13	0.019
		*1e																							6	0.0088
		*1f																							6	0.0088
		*1g																							3	0.0044
		*1h																							2	0.0029
		*1i																							2	0.0029
		*1j																							2	0.0029
		*1k																							1	0.0015
		*1l																							1	0.0015
	*5	*5a																							15	0.022
		*5b																							4	0.0059
		*5c$																							1	0.0015
	*11	*11a																							1	0.0015
	*166V	*166Va																							8	0.012
		*166Vb																							3	0.0044
	*151D	*151D																							6	0.0088
	*109N	*109N																							2	0.0029
	*13E	*13E																							1	0.0015
	*265K	*265K																							1	0.0015
	*245F	*245F$																							1	0.0015

b) Block 2

Nucleotide change			IVS12-11G>A	IVS12-9A>G	1543G>A	1572T>G	1627A>G	1666A>C	IVS13+39 C>T	IVS13+40 G>A	Number	Frequency
Amino acid change					V515I	F524L	I543V	S556R				
Haplotype	*1	*1a									361	0.529
		*1b									120	0.176
		*1c									5	0.0073
	*5	*5a					5a				167	0.245
		*5b					5b				26	0.038
	*515I	*515I			515I						1	0.0015
	*524L	*524L				524L					1	0.0015
	*556R	*556R$						556R			1	0.0015

c) Block 3

Nucleotide change			IVS13-47_48insTA	1752A>G	1774C>T	1896T>C	IVS14+19 C>A	IVS14+100 T>G	Number	Frequency
Amino acid change				T584T	R592W	F632F				
Haplotype	*1	*1a							578	0.848
		*1b							94	0.138
		*1c							6	0.0088
		*1d							1	0.0015
		*1e							1	0.0015
		*1f$							1	0.0015
	*592W	*592W			592W				1	0.0015

d) Block 4

Nucleotide change			IVS14-123C>A	IVS14-21C>A	IVS15+75 A>G	Number	Frequency
Amino acid change							
Haplotype	*1	*1a				576	0.845
		*1b				105	0.154
		*1c$				1	0.0015

e) Block 5

Nucleotide change			IVS16-127A>G	IVS16-94G>T	IVS17+34delT	IVS17+47C>T	2194G>A	Number	Frequency
Amino acid change							V732I		
Haplotype	*1	*1a						414	0.607
		*1b						255	0.374
		*1c$						1	0.0015
		*1d$						1	0.0015
		*1e$						1	0.0015
	*6	*6a					6a	10	0.015

f) Block 6

Nucleotide change			IVS18-39G>A	2303C>A	2424T>C	2678A>G	IVS21+80 C>G	IVS21+113 T>A	IVS21+136 G>C	IVS21+162 T>G	IVS22+129 A>G	IVS22-69 G>A	IVS22-58 G>C	Number	Frequency
Amino acid change				T768K	S808S	N893S									
Haplotype	*1	*1a												624	0.915
		*1b												22	0.032
		*1c												5	0.0073
		*1d												2	0.0029
		*1e												2	0.0029
		*1f												2	0.0029
		*1g												2	0.0029
		*1h												1	0.0015
		*1i												1	0.0015
	*768K	*768K		768K										19	0.028
	*893S	*893S				893S								2	0.0029

Fig. 3 Block haplotypes in *DPYD* of block 1 (a), block 2 (b), block 3 (c), block 4 (d), block 5 (e), and block 6 (f) in a Japanese population. The nucleotide positions were numbered based on the cDNA sequence (A of the translational start codon is +1) or from the nearest exon. *White cell* wild-type, *gray cell* nucleotide alteration. $The haplotypes were inferred in only one patient and ambiguous except for marker SNPs

Table 3 Linkages of haplotype-tagging SNPs with HapMap SNPs for *DPYD*

Haplotype-tagging SNPs in *DPYD*	dbSNP ID (NCBI)	Block haplotype in this paper	HapMap SNPs with close linkages ($r^2 > 0.8$)[a]
85T>C (Cys29Arg)	rs1801265	Block 1 *#9*	rs10747488, rs7526108, rs4421623, rs4379706, rs4523551,rs11165921, rs9661794, rs6677116, rs6604093, rs17379561, rs10747491, rs10747492, rs12062845, rs7524038, rs10875112, rs4394693, rs10875113, rs4970722, rs9727548, rs10875118, rs9662719, rs12077442, rs4394694, rs9727976, rs4246515, rs6692580
496A>G (Met166Val)	rs2297595	Block 1 *#166V*	rs2786543, rs2811215, rs2811214, rs2786544, rs2248658, rs11165897, rs2786490, rs2811203, rs2811202, rs2811200, rs2811198, rs2786503, rs2811196, rs2786505, rs2811195, rs2811194, rs12073839, rs6663670, rs7512910, rs2151563, rs2786509, rs3790387, rs3790389
1627A>G (Ile543Val)	rs1801159	Block 2 *#5*	rs1415682, rs952501, rs2811187, rs2786778, rs2786774, rs2811183, rs17116806, rs2786780, rs1801159, rs2786771, rs2297780, rs2297779, rs12729863
1896T>C (Phe632Phe)	rs7556439	Block 3 *#1b*	rs12073650
IVS16-94G>T	rs7556439	Block 5 *#1b*	rs693680, rs827500, rs499009, rs7518848, rs553388, rs507170, rs628959, rs991544, rs526645, rs1609519
IVS18-39G>A	rs12137711	Block 6 *#1b*	rs12120068, rs12116905

[a] All SNPs are in the same block

Taken together, our data demonstrated considerable differences in the haplotype distributions in blocks 1, 3 and 6 between Japanese and Caucasians.

Discussion

This study provides Japanese data on the genetic variations of *DPYD*, a gene encoding a key enzyme catalyzing degradation of the well-known anticancer drug 5-FU. Nine novel (Ala10Glu, Tyr109Asn, Asn151Asp, Ile245Phe, Glu265Lys, Val515Ile, Phe524Leu, Ser556Arg, and Asn893Ser) and seven known nonsynonymous variations (Cys29Arg, Met166Val, Val335Leu, Ile543Val, Arg592Trp, Val732Ile, and Thr768Lys) were found in our Japanese population (Table 2 and Fig. 1). The association analysis between the genotypes and 5-FU pharmacodynamics is now on-going.

Uneven distributions of coding SNPs over 23 *DPYD* exons were pointed out in the previous review by van Kuilenburg (2004). The author indicated that 81% of all reported variations were confined to exons 2–14, representing 61% of the coding sequences, and typical hotspots of variation were localized in exons 2, 6, and 13. Our Japanese data also revealed that 17 out of 21 coding variations (81%) were localized in exons 1–14, and that more than three variations were detected in exons 5, 13, and 14 (Fig. 1). Recently, Hormozian et al. (2007) have reported that the common chromosomal fragile site on 1p21.2, *FRA1E*, spans 370 kb of genomic sequence between introns 8 and 18 of *DPYD*, and that its core region with the highest fragility is located between introns 12 and 16. The instability at the core of *FRA1E* might be associated with the high mutational rates and recombinogenic nature from intron 12 to 14 of *DPYD* (Fig. 1).

To estimate potential functional consequences of the amino acid substitutions, we examined whether the positions of amino acid changes are located in highly conserved areas or potentially critical regions of the molecule (for example, substrate recognition sites or binding regions of prosthetic groups). We also considered the locations of the residues in a three-dimensional (3D) framework provided by the crystal structures of pig DPD, which have recently been determined in complexes with NADPH and substrate (5-FU) (Dobritzsch et al. 2001) or inhibitors (Dobritzsch et al. 2002). The amino acid sequences of pig and human DPD are 93% identical (Mattison et al. 2002), and the substituted residues and their neighboring residues are conserved between both enzymes. From these points of view, it is speculated that at least two substitutions (Glu265Lys and Arg592Trp) might impact the structure and function of DPD as discussed below.

Glu265 is located on the loop following to the third β sheet (IIβ3) in the FAD binding domain II (Dobritzsch et al. 2001). Glu265 is conserved among four mammalian species (human, mouse, rat, and pig), although it is replaced with aspartic acid in bovine and *Drosophila melanogaster* DPDs (Mattison et al. 2002). In the 3D structure of pig DPD (Fig. 5a), Glu265 is in close proximity to Lys259. The substitution, Lys259Glu, was

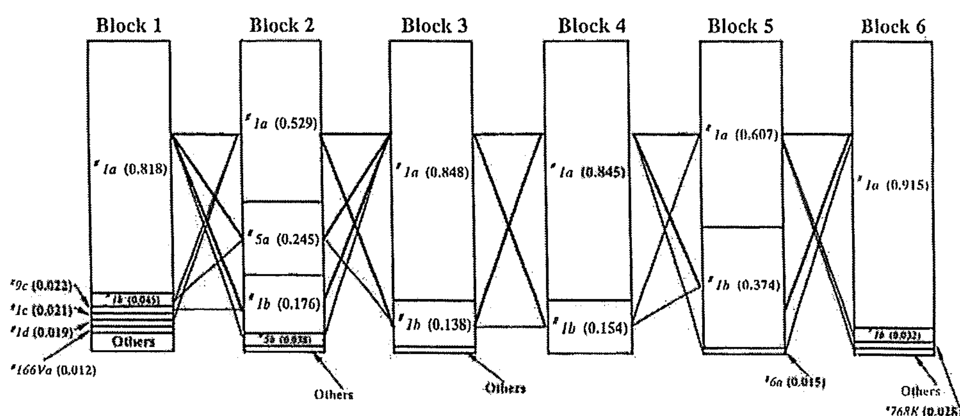


Fig. 4 The combinations of block haplotypes in Japanese. *Thick lines* represent combinations with frequencies over 10%, and *thin lines* represent combinations with frequencies of 1.0–9.9%

detected in the patient exhibiting severe mucositis during cyclophosphamide/methotrexate/5-FU chemotherapy (Gross et al. 2003). Furthermore, the adjacent Leu261 interacts via the main chain atoms with the N6, N1, and N3 atoms of adenine of FAD, and has an important role in the proper orientation of the adenine moiety in the FAD-binding pocket (Dobritzsch et al. 2001). Moreover, the carboxyl group (Glu265-Oε)might form hydrogen bonds to the main chain nitrogen of Ser260 next to Leu261. Thus, the change in polarity from negative to positive by the novel Glu265Lys substitution is likely to cause structural changes affecting proper binding of FAD.

Arg592 is located at one (IVβc) of the additional four-stranded antiparallel β sheets (IVβc-βf) inserted at the top of a typical $(\alpha/\beta)_8$ barrel fold in the FMN-binding domain IV (Dobritzsch et al. 2001). Arg592 is completely conserved among the above-mentioned six species (Mattison et al. 2002), suggesting its functional importance. Arg592 closely contacts Met599 (2.9 Å) and Gln604 (2.8 Å) in the same subunit and Ser994 (2.9 Å) in another subunit (Fig. 5B). The substitution of tryptophan for Arg592 is likely to weaken these interactions due to altered hydrophobicity and electrostatic changes. Arg592Trp was recently reported from a Korean population with an allele frequency of 0.004, although its functional significance remains to be confirmed(Cho et al. 2007).

As for known *DPYD* alleles, their distributions in several populations are becoming more evident by recent reports. For example, IVS14 + 1G>A (*2) (van Kuilenburg 2004), 295_298delTCAT (Phe100SerfsX15, *7) (Seck et al. 2005), 1679T>G (Ile560Ser, *13) (Collie-Duguid et al. 2000; Morel et al. 2006) 2846A>T (Asp949Val) (Seck et al. 2005; Morel et al. 2006), all of which are associated with decreased DPD activities, are detected in Caucasians with allele frequencies of 0.01–0.02, 0.003, 0.001 and 0.006–0.008, respectively. However, none of them were detected in our Japanese samples, while 1003G>T (Val335Leu, *11) and 2303C>A (Thr768Lys) have been found only in Japanese, indicating that variations with clinical relevance do not overlap between Caucasians and Japanese.

2303C>A (Thr768Lys), which was originally found in a Japanese female volunteer with very low DPD activity (Ogura et al. 2005), is relatively frequent in Japanese (allele frequency = 0.0279). Functional characterization in vitro revealed that 768Lys caused thermal instability of the variant protein without changing its affinity for NADPH or kinetic parameters toward 5-FU. Therefore, they might cause 5-FU-related toxicities in Japanese.

1003G>T (Val335Leu, *11) was found in a Japanese family with decreased DPD activity by Kouwaki et al. (1998). By in vitro expression in *E. coli*, they demonstrated that the variant protein with Leu335 showed a significant loss of activity (about 17% of the wild-type protein). Dobritzsch et al. (2001) suggested from the 3D structure of pig DPD that Val335Leu, in spite of a conservative change, disturbs packing interactions in the hydrophobic core formed by IIIβ3 and IIIα3 within the Rossman-motif, thereby affecting NADPH binding. In our study, heterozygous 1003G>T (Val335Leu) was found from a patient administrated 5-FU (allele frequency = 0.0015), who also has seven other variations: IVS12–11G>A, 1896T>C (Phe632Phe), and IVS16–94G>T are heterozygous, and 1627A>G (Ile543Val), IVS13 + 39C>T, IVS14–123C>A, and IVS15 + 75A>G are homozygous, indicating that at least Val335Leu is linked to Ile543Val (*5).

On the other hand, Caucasians and Japanese share four variations: *5 (Ile543Val), *9 (Cys29Arg), Met166Val, and *6 (Val732Ile), although their allele frequencies were different, especially for *9 (Table 4). Because they have not necessarily correlated with phenotypic changes (e.g., differences in DPD enzyme activity, 5-FU pharmacokinetics and pharmacodynamics) (Collie-Duguid et al. 2000; Johnson et al. 2002; Zhu et al. 2004; Seck et al. 2005; Ridge et al. 1998a, 1998b; Hsiao et al. 2004), all of these variations are generally accepted as common polymorphisms that result in unaltered function. Consistent with this, van Kuilenburg et al. (2002) suggested that the

Table 4 Allele frequencies of common *DPYD* SNPs in different populations

Nucleotide change (amino acid change)	Allele or tagged haplotypes	Population	Allele frequency	Number of subjects	Reference
85T>C	*9	Caucasian	0.194	157	Seck et al. 2005
(Cys29Arg)	(Block 1 #9)	French Caucasian	0.185	487	Morel et al. 2006
		Japanese	0.037	107	Yamaguchi et al. 2001
		Japanese	0.029	341	This study
		Taiwanese	0.022	300	Hsiao et al. 2004
496A>G	Block 1 #166V	Caucasian	0.080	157	Seck et al. 2005
(Met166Val)		Japanese	0.022	341	This study
IVS10-15T>C	Block 1 #166Va, #9d	Caucasian	0.127	157	Seck et al. 2005
		Japanese	0.018	341	This study
1627A>G	*5	Caucasian	0.140	157	Seck et al. 2005
(Ile543Val)	(Block 2 #5)	Caucasian	0.275	60	Ridge et al. 1998a
		Finnish	0.072	90	Wei et al. 1998
		African-American	0.227	105	Wei et al. 1998
		Japanese	0.352	50	Wei et al. 1998
		Japanese	0.283	341	This study
		Taiwanese	0.210	131	Wei et al. 1998
		Taiwanese	0.283	300	Hsiao et al. 2004
1896T>C	Block 3 #1b	Caucasian	0.035	157	Seck et al. 2005
(Phe632Phe)		Japanese	0.098	107	Yamaguchi et al. 2001
		Japanese	0.139	341	This study
		Han Chinese	0.133	45	HapMap
IVS15 + 75A>G	Block 4 #1b	Caucasian	0.166	157	Seck et al. 2005
		Japanese	0.155	341	This study
IVS16-94G>T	Block 5 #1b	Caucasian	0.415	59	HapMap
		Yorba	ND	60	HapMap
		Japanese	0.455	44	HapMap
		Japanese	0.378	341	This study
		Han Chinese	0.333	45	HapMap
2194G>A	*6	Caucasian	0.022	157	Seck et al. 2005
(Val732Ile)	(Block 5 #6)	Caucasian	0.058	60	Ridge et al. 1998a
		Finnish	0.067	90	Wei et al. 1998
		African-American	0.019	105	Wei et al. 1998
		Japanese	0.044	50	Wei et al. 1998
		Japanese	0.015	341	This study
		Taiwanese	0.014	131	Wei et al. 1998
		Taiwanese	0.012	300	Hsiao et al. 2004
IVS18-39G>A	Block 6 #1b	Caucasian	0.105	157	Seck et al. 2005
		Caucasian	0.100	60	HapMap
		Yorba	0.017	60	HapMap
		Japanese	0.044	45	HapMap
		Japanese	0.032	341	This study
		Han Chinese	0.022	45	HapMap
IVS22-69G>A	Block 6 #1f	Caucasian	0.183	60	HapMap
		Yorba	0.400	60	HapMap
		Japanese	ND	45	HapMap
		Japanese	0.003	341	This study
		Han Chinese	ND	45	HapMap

ND not detected

substitution Cys29Arg on the protein surface was unlikely to alter DPD activity. However, conflicting results were reported regarding *9 (Vreken et al. 1997, van Kuilenburg et al. 2000), *6 (van Kuilenburg et al. 2000), and Met166Val (van Kuilenburg et al. 2000; Gross et al. 2003). To interpret these inconsistencies, haplotype analysis of *DPYD* might be helpful. Especially for *9 and Met166Val in Japanese, functional involvement of −477T>G (block 1 #9c and #9e), −243G>A (block 1 #9d), IVS10-15T>C (block 1 #9d and #166Va) and many other HapMap SNPs linked to *9 and Met166Val (Table 3) needs clarification.

The HapMap project provides genotype data of more than 1,000 sites located mostly in the intronic regions of *DPYD* for four different populations (Nigerian, Chinese,

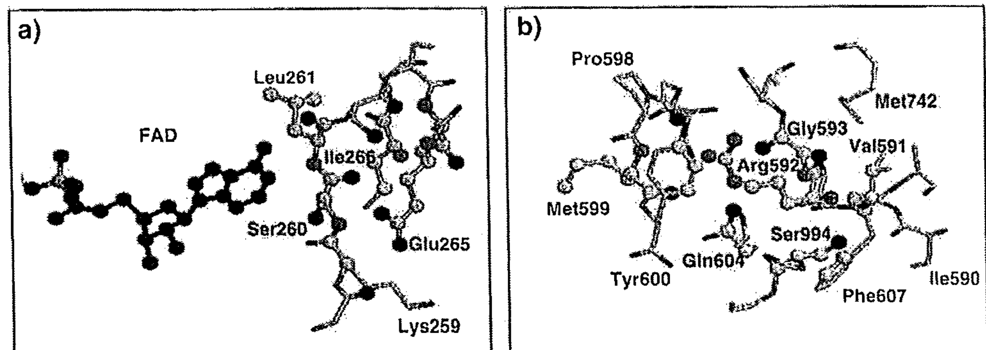


Fig. 5 Stereo view of the variation sites in pig DPD (accession code of the Protein Data Bank: 1gth). Glu265 (**a**), Arg592 (**b**) and their adjacent residues are shown as *ball-and-stick* models with oxygens in *red*, nitrogens in *blue*, carbons in *gray* and sulfur in *yellow*. The adenosine moiety of the cofactor FAD is also shown in *pink* (**a**)

Japanese and Caucasians). HapMap data on 44 unrelated Japanese subjects showed that 476 variations are polymorphic, whereas 529 are monomorphic, and the average density of polymorphic markers is 1 SNP per 1,772 bp. In contrast, our study focused on exons and surrounding introns to detect variations, and only nine variations overlapped with the HapMap data. Therefore, we could not utilize the HapMap data to further identify common subtypes of *#1* to be discriminated by many intronic HapMap SNPs in each block. However, most of the frequent SNPs are unlikely to be associated with substantially decreased DPD activity because DPD activity in the healthy Japanese population (N = 150) showed a unimodal Gaussian distribution (Ogura et al. 2005).

On the other hand, in 60 unrelated Caucasian subjects in the HapMap project, 617 are polymorphic, whereas 383 are monomorphic. LD profiles of these polymorphisms were compared between Caucasians and Japanese by using the program Marker (http://www.gmap.net/marker). Strong LD (|D'| > 0.75) clearly decays within introns 11, 12, 13, 14, 16, 18, and 20 in Japanese, whereas, similar decays are observed within introns 13, 14, 18, and 20, but are not obvious within introns 11, 12, and 16 in Caucasians (data not shown). Moreover, strong LD decays within intron 3 in Caucasians. Therefore, the LD blocks are considerably different between Japanese and Caucasians. Along with the marked differences in allele frequencies of several variations (Table 4), these results suggest that the haplotype structures in *DPYD* are quite different between the two populations.

In conclusion, we found 55 variations, including 38 novel ones, in *DPYD* from 341 Japanese subjects. Nine novel nonsynonymous SNPs were found, some of which were assumed to have impact on the structure and function of DPD. As for known variations, we obtained their accurate allele frequencies in a Japanese population of a large size and showed that variations with clinical relevance do not overlap between Caucasians and Japanese. In Japanese, 2303C>A (Thr768Lys) and 1003G>T (Val335Leu) might play important roles in 5-FU-related toxicity. Along with differences in haplotype structures between Japanese and Caucasians, these findings suggest that ethnic-specific tagging SNPs should be considered on genotyping *DPYD*. Thus, the present information would be useful for pharmacogenetic studies for evaluating the efficacy and toxicity of 5-FU in Japanese and probably in East Asians.

Acknowledgments We thank Ms. Chie Sudo for her secretarial assistance. This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences (05–25) of the National Institute of Biomedical Innovation and in part by the Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare.

References

Bakkeren JA, De Abreu RA, Sengers RC, Gabreels FJ, Maas JM, Renier WO (1984) Elevated urine, blood and cerebrospinal fluid levels of uracil and thymine in a child with dihydrothymine dehydrogenase deficiency. Clin Chim Acta 140:247–256

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Cho HJ, Park YS, Kang WK, Kim JW, Lee SY (2007) Thymidylate synthase (TYMS) and dihydropyrimidine dehydrogenase (DPYD) polymorphisms in the Korean population for prediction of 5-fluorouracil-associated toxicity. Ther Drug Monit 29:190–196

Collie-Duguid ES, Etienne MC, Milano G, McLeod HL (2000) Known variant DPYD alleles do not explain DPD deficiency in cancer patients. Pharmacogenetics 10:217–223

Dobritzsch D, Schneider G, Schnackerz KD, Lindqvist Y (2001) Crystal structure of dihydropyrimidine dehydrogenase, a major determinant of the pharmacokinetics of the anti-cancer drug 5-fluorouracil. Embo J 20:650–660

Dobritzsch D, Ricagno S, Schneider G, Schnackerz KD, Lindqvist Y (2002) Crystal structure of the productive ternary complex of dihydropyrimidine dehydrogenase with NADPH and 5-iodouracil. Implications for mechanism of inhibition and electron transfer. J Biol Chem 277:13155–13166

Etienne MC, Lagrange JL, Dassonville O, Fleming R, Thyss A, Renee N, Schneider M, Demard F, Milano G (1994) Population study of dihydropyrimidine dehydrogenase in cancer patients. J Clin Oncol 12:2248–2253

Gross E, Ullrich T, Seck K, Mueller V, de Wit M, von Schilling C, Meindl A, Schmitt M, Kiechle M (2003) Detailed analysis of five mutations in dihydropyrimidine dehydrogenase detected in

cancer patients with 5-fluorouracil-related side effects. Hum Mutat 22:498

Grem JL (1996) Fluoropyrimidines. In: Chabner BA, Longo DL (eds) Cancer chemotherapy and biotherapy, 2nd edn. Lippincott-Raven, Philadelphia, pp 149–197

Heggie GD, Sommadossi JP, Cross DS, Huster WJ, Diasio RB (1987) Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. Cancer Res 47:2203–2206

Hormozian F, Schmitt JG, Sagulenko E, Schwab M, Savelyeva L (2007) *FRA1E* common fragile site breaks map within a 370 kilobase pair region and disrupt the dihydropyrimidine dehydrogenase gene (*DPYD*). Cancer Lett 246:82–91

Hsiao HH, Yang MY, Chang JG, Liu YC, Liu TC, Chang CS, Chen TP, Lin SF (2004) Dihydropyrimidine dehydrogenase pharmacogenetics in the Taiwanese population. Cancer Chemother Pharmacol 53:445–451

Johnson MR, Wang K, Diasio RB (2002) Profound dihydropyrimidine dehydrogenase deficiency resulting from a novel compound heterozygote genotype. Clin Cancer Res 8:768–774

Kitamura Y, Moriguchi M, Kaneko H, Morisaki H, Morisaki T, Toyama K, Kamatani N (2002) Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. Ann Hum Genet 66: 183–193

Kouwaki M, Hamajima N, Sumi S, Nonaka M, Sasaki M, Dobashi K, Kidouchi K, Togari H, Wada Y (1998) Identification of novel mutations in the dihydropyrimidine dehydrogenase gene in a Japanese patient with 5-fluorouracil toxicity. Clin Cancer Res 4:2999–3004

Lu Z, Zhang R, Diasio RB (1993) Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients, and clinical implication in 5-fluorouracil chemotherapy. Cancer Res 53:5433–5438

Lu Z, Zhang R, Carpenter JT, Diasio RB (1998) Decreased dihydropyrimidine dehydrogenase activity in a population of patients with breast cancer: implication for 5-fluorouracil-based chemotherapy. Clin Cancer Res 4:325–329

Martz E (2002) Protein explorer: easy yet powerful macromolecular visualization. Trends Biochem Sci 27:107–109

Mattison LK, Johnson MR, Diasio RB (2002) A comparative analysis of translated dihydropyrimidine dehydrogenase cDNA; conservation of functional domains and relevance to genetic polymorphisms. Pharmacogenetics 12:133–144

McLeod HL, Collie-Duguid ES, Vreken P, Johnson MR, Wei X, Sapone A, Diasio RB, Fernandez-Salguero P, van Kuilenberg AB, van Gennip AH, Gonzalez FJ (1998) Nomenclature for human DPYD alleles. Pharmacogenetics 8:455–459

Morel A, Boisdron-Celle M, Fey L, Soulie P, Craipeau MC, Traore S, Gamelin E (2006) Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. Mol Cancer Ther 5:2895–2904

Naguib FN, el Kouni MH, Cha S (1985) Enzymes of uracil catabolism in normal and neoplastic human tissues. Cancer Res 45:5405–5412

Nishiyama T, Ogura K, Okuda H, Suda K, Kato A, Watabe T (2000) Mechanism-based inactivation of human dihydropyrimidine dehydrogenase by (E)-5-(2-bromovinyl)uracil in the presence of NADPH. Mol Pharmacol 57:899–905

Ogura K, Ohnuma T, Minamide Y, Mizuno A, Nishiyama T, Nagashima S, Kanamaru M, Hiratsuka A, Watabe T, Uematsu T (2005) Dihydropyrimidine dehydrogenase activity in 150 healthy Japanese volunteers and identification of novel mutations. Clin Cancer Res 11:5104–5111

Ridge SA, Sludden J, Brown O, Robertson L, Wei X, Sapone A, Fernandez-Salguero PM, Gonzalez FJ, Vreken P, van Kuilenburg AB, van Gennip AH, McLeod HL (1998a) Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. Br J Clin Pharmacol 46:151–156

Ridge SA, Sludden J, Wei X, Sapone A, Brown O, Hardy S, Canney P, Fernandez-Salguero P, Gonzalez FJ, Cassidy J, McLeod HL (1998b) Dihydropyrimidine dehydrogenase pharmacogenetics in patients with colorectal cancer. Br J Cancer 77:497–500

Seck K, Riemer S, Kates R, Ullrich T, Lutz V, Harbeck N, Schmitt M, Kiechle M, Diasio R, Gross E (2005) Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in a cohort of Caucasian individuals. Clin Cancer Res 11:5886–5892

Shestopal SA, Johnson MR, Diasio RB (2000) Molecular cloning and characterization of the human dihydropyrimidine dehydrogenase promoter. Biochim Biophys Acta 1494:162–169

van Kuilenburg AB (2004) Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. Eur J Cancer 40:939–950

van Kuilenburg AB, Haasjes J, Richel DJ, Zoetekouw L, Van Lenthe H, De Abreu RA, Maring JG, Vreken P, van Gennip AH (2000) Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. Clin Cancer Res 6:4705–4712

van Kuilenburg AB, Dobritzsch D, Meinsma R, Haasjes J, Waterham HR, Nowaczyk MJ, Maropoulos GD, Hein G, Kalhoff H, Kirk JM, Baaske H, Aukett A, Duley JA, Ward KP, Lindqvist Y, van Gennip AH (2002) Novel disease-causing mutations in the dihydropyrimidine dehydrogenase gene interpreted by analysis of the three-dimensional protein structure. Biochem J 364:157–163

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

Wei X, Elizondo G, Sapone A, McLeod HL, Raunio H, Fernandez-Salguero P, Gonzalez FJ (1998) Characterization of the human dihydropyrimidine dehydrogenase gene. Genomics 51:391–400

Yamaguchi K, Arai Y, Kanda Y, Akagi K (2001) Germline mutation of dihydropyrimidine dehydrogenese gene among a Japanese population in relation to toxicity to 5-Fluorouracil. Jpn J Cancer Res 92:337–342

Zhang K, Qin Z, Chen T, Liu JS, Waterman MS, Sun F (2005) HapBlock: haplotype block partitioning and tag SNP selection software using a set of dynamic programming algorithms. Bioinformatics 21:131–134

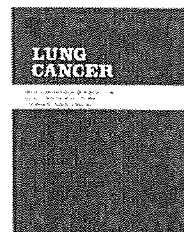
Zhu AX, Puchalski TA, Stanton VP Jr, Ryan DP, Clark JW, Nesbitt S, Charlat O, Kelly P, Kreconus E, Chabner BA, Supko JG (2004) Dihydropyrimidine dehydrogenase and thymidylate synthase polymorphisms and their association with 5-fluorouracil/leucovorin chemotherapy in colorectal cancer. Clin Colorectal Cancer 3:225–234

Lung Cancer (2007) 58, 362–368

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/lungcan



Randomized phase II trial of three intrapleural therapy regimens for the management of malignant pleural effusion in previously untreated non-small cell lung cancer: JCOG 9515

Kimihide Yoshida[a,*], Takahiko Sugiura[a], Nobuhide Takifuji[b], Masaaki Kawahara[c], Kaoru Matsui[d], Shinzoh Kudoh[e], Minoru Takada[f], Masahiro Fukuoka[g], Yutaka Ariyoshi[h], Haruhiko Fukuda[i], Nagahiro Saijo[j]

[a] *Department of Thoracic Oncology, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan*
[b] *Department of Medical Oncology, Osaka City General Hospital, Osaka, Japan*
[c] *Department of Internal Medicine, National Kinki Chuo Hospital, Osaka, Japan*
[d] *Department of Internal Medicine, Osaka Prefectural Habikino Hospital, Habikino, Japan*
[e] *Department of Internal Medicine, Osaka City University, Osaka, Japan*
[f] *Department of Pulmonary Diseases, Rinku Genaral Hospital, Izumisano, Japan*
[g] *Department of Medical Oncology, Kinki University, Sayama, Japan*
[h] *Department of Internal Medicine, Aichi Prefectural Aichi Hospital, Okazaki, Japan*
[i] *Japan Clinical Oncology Group Data Center, National Cancer Center, Tokyo, Japan*
[j] *National Cancer Center Hospital East, Kashiwa, Japan*

Received 10 May 2007; received in revised form 9 July 2007; accepted 15 July 2007

KEYWORDS
Non-small cell lung cancer;
Malignant pleural effusion;
Intrapleural therapy;
Management of malignant pleural effusion;
Bleomycin;
OK-432;
Cisplatin plus etoposide

Summary To evaluate the efficacy and toxicity of three intrapleural therapy regimens consisting of bleomycin (BLM), OK-432 (a pulverized product of heat-killed *Streptococcus pyogenes*) or cisplatin plus etoposide (PE) for the management of malignant pleural effusion (MPE) in previously untreated non-small cell lung cancer. Eligible patients were randomized to the BLM arm: BLM 1 mg/kg (maximum 60 mg/body), the OK-432 arm: OK-432 0.2 Klinische Einheit units (KE)/kg (maximum 10 KE/body), or the PE arm: cisplatin (80 mg/m^2) and etoposide (80 mg/m^2). Pleural response was evaluated every 4 weeks according to the study-specific criteria. All responders received systemic chemotherapy consisting of PE every 3–4 weeks for two or more courses. Pleural progression-free survival (PPFS) was defined as the time from randomization to the first observation of pleural progression or death due to any cause. The primary endpoint was the 4-week PPFS rate. Of 105 patients enrolled, 102 were assessed for response. The 4-week PPFS rate for the BLM arm was 68.6%, 75.8% for the OK-432 arm, and 70.6% for PE arm. Median survival time (MST) for the BLM arm was 32.1 weeks, 48.1 weeks for the OK-432 arm, and 45.7 weeks

* Corresponding author. Tel.: +81 52 762 6111; fax: +81 52 763 5233.
E-mail address: 105197@aichi-cc.jp (K. Yoshida).

0169-5002/$ — see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2007.07.009

for the PE arm. However, the outcomes did not differ significantly between groups. Toxicity was tolerable in all arms except for one treatment-related death due to interstitial pneumonia induced by BLM. We will select intrapleural treatment using OK-432 in the management of MPE in NSCLC for further investigation because it had the highest 4-week PPFS rate.
© 2007 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Malignant pleural effusion (MPE) is a significant problem in the treatment of patients with advanced malignancies and is a major cause of poor prognosis [1]. The most widely used therapy for MPE is tube drainage with intrapleural instillation of sclerosing agents to prevent fluid reaccumulation [2].

Despite many reported trials of chemical pleurodesis, there has been no agreement as to the optimal treatment protocol for MPE [3–5]. The variety of response rates of individual agents among those studies has resulted from heterogeneous patient populations and differences in treatment procedures and response criteria [2,3,6]. To resolve these problems, we conducted a randomized phase II trial in which patient selection was limited to previously untreated patients with MPE due to non-small cell lung cancer (NSCLC) and, in view of adequate estimation of the efficacy of each intrapleural therapy regimen, single instillation of chemical agents and uncomplicated study-specific response criteria were applied. In this study, to select the most promising regimen for intrapleural therapy consisting of sclerosing or chemotherapeutic agents, we chose three regimens—BLM, OK-432 and cisplatin plus etoposide (PE). BLM was chosen because it is one of the most frequently used agents and is considered to have high efficacy, low toxicity and high availability [3,5,7,8]. OK-432 (a preparation of Streptococcus pyogenes, type A3, Chugai Pharmaceutical Co., Tokyo) has been used as an anti-tumor immunomodulator for lung cancer [9,10] and is reported to give superior responses for MPE compared to mitomycin C [11] and BLM [12]. At the beginning of this study, PE regimens were considered one of the standard combination chemotherapy regimens for NSCLC, and a phase II trial using this regimen for intrapleural therapy suggested potential survival benefit as well as local control effects [13].

2. Methods

2.1. Patient selection

The eligibility criteria were as follows: cytologically or histologically proven malignant pleural effusion associated with newly diagnosed NSCLC; no prior chemotherapy, thoracic radiotherapy or thoracic surgery; age of 75 years or less; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2 after tube thoracostomy; full lung reexpansion after tube thoracostomy; adequate bone marrow reserve (WBC count $\geq 4000\,\mu L^{-1}$, hemoglobin ≥ 9.5 g/dL, and platelet count $\geq 100{,}000\,\mu L^{-1}$), and liver (total bilirubin ≤ 1.5 mg/dL and transaminase levels $\leq$ twice the upper limit of the normal value) and renal (BUN ≤ 25 mg/dL, serum creatinine ≤ 1.2 mg/dL, and creatinine clearance ≥ 50 mL/min) functions. All patients gave written, informed consent, and the protocol and the consent form were approved by the Clinical Trial Review Committee of the Japan Clinical Oncology Group (JCOG) and by the institutional review boards of all participating institutions.

The exclusion criteria were bilateral pleural effusion or pericardial effusion, symptomatic brain metastases requiring whole-brain irradiation or administration of corticosteroids, an active synchronous cancer, interstitial pneumonitis, pulmonary fibrosis, uncontrolled angina pectoris or myocardial infarction within the preceding 3 months, uncontrolled diabetes mellitus or hypertension, pregnancy or breast-feeding, and penicillin allergy.

2.2. Treatment and monitoring

All patients were required to have either large-bore chest tubes or small-bore catheters placed, with radiographic evidence of reexpansion of the affected lung following suction or gravity drainage. Patients were stratified by institution and PS after tube drainage and then randomly assigned to the three treatment groups (Fig. 1). Intrapleural therapy was performed as follows. In the BLM and OK-432 arms, following instillation of either BLM (1 mg/kg, maximum 60 mg/body) or OK-432 (0.2 Klinische Einheit units (KE)/kg, maximum 10 KE/body), diluted in 100 ml of physiologic saline, the tube was clamped and the patient's position rotated for 3 h. Then the tube was unclamped and allowed to drain. In the PE arm, cisplatin (80 mg/m^2) and etoposide (80 mg/m^2) diluted in 100 ml of physiologic saline were simultaneously administered into the pleural cavity, the tube was clamped and the patient's position rotated for 3 h. Seventy-two hours later, the tube was unclamped and allowed to drain.

The tube was removed when the pleural effusion decreased to 100 ml or less per day. If more than 100 ml of drained fluid continued for 7 days or the pleural effusion increase by chest radiographs within 4 weeks, the patient was taken off the protocol and considered as a treatment failure.

2.3. Response criteria

The response criteria used were (i) response—disappearance or residual effusion with no need of local treatment (no greater than one quarter of the treated lung field nor remarkable increase compared to baseline chest radiographs) and (ii) pleural progression—a greater than one quarter of the treated lung field increase in pleural effusion compared to baseline chest radiographs.

2.4. Response evaluation and systemic chemotherapy

Pleural response was evaluated at the 4th, 8th, 12th and 24th week according to the study-specific criteria (see

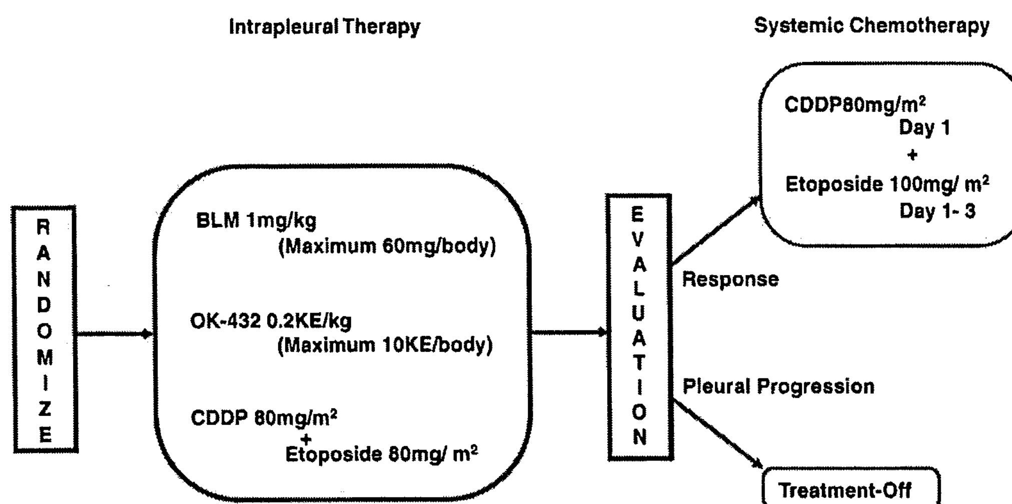


Fig. 1 Treatment schema.

above). A responder identified within 2 weeks after the first (4-week) evaluation received systemic chemotherapy consisting of cisplatin (80 mg/m^2) on day 1 and etoposide (100 mg/m^2) on days 1—3, which was repeated every 3—4 weeks for two or more courses.

2.5. Toxicity criteria and dose modification

Adverse reactions were graded according to the JCOG Toxicity Criteria [14], which are modifications of the National Cancer Institute's common toxicity criteria issued in 1991. The second or subsequent cycles of systemic chemotherapy were delayed if on day 1 the WBC count was less than 3000 μL^{-1} or the platelet count was less than 75,000 μL^{-1}. If grade 4 hematological toxicity occurred during the previous course, the dose of etoposide was reduced to 75%. Cisplatin was permanently discontinued at any time when the serum creatinine level was greater than 2.0 mg/dL. If the serum creatinine level was 1.5—2.0 mg/dL, the next cycle was delayed until it was 1.2 mg/dL or less, and the dose of cisplatin was then reduced to 75%.

2.6. Data management and statistical analysis

This study was designed as a multicenter randomized phase II trial among 21 participating centers in the Lung Cancer Study Group in the JCOG. Pleural progression-free survival (PPFS) was defined as the time from randomization to the first observation of pleural progression or death due to any cause. The primary endpoint of this study was 4-week PPFS rate. Assuming that the 4-week PPFS rate was at least 50% for these arms, the required number for each arm was 30 to select the better arm correctly with 90% probability if the better arm's 4-week PPFS rate was 70% or higher [15]. Planned accrual was set at 35 per arm. Secondary end-points were 8-, 12- and 24-week PPFS rates, overall survival (OS) and toxicity. The duration for OS was measured from the date of randomization to the date of death due to any cause or last follow-up. The mandated time to start treatment following randomization was within a week. Survival distribution was estimated by the Kaplan—Meier method, and confidence intervals were based on Greenwood's formula [16].

Patient randomization and data management were performed by the JCOG Data Center (JCOG DC). In-house interim monitoring was performed by the JCOG Data and Safety Monitoring Committee semiannually. Central review of chest X-rays for all responses in all eligible cases was performed at regular study group meetings by an extramural panel. Statistical analysis was performed by the JCOG DC with SAS software version 6.12 for Windows (SAS Institute Inc., Cary NC).

3. Results

3.1. Patients

From May 1996 to August 1999, 105 patients were enrolled onto this study from the 21 participating institutions. The clinical characteristics of the patients are listed in Table 1. Three patients were later found to be ineligible (one patient per group): one had malignant pleural effusion secondary to colon cancer; one had no reexpansion of the affected lung after tube drainage; and one had poor renal function. Thus, 102 patients were assessable for response and survival. Four patients did not receive intrapleural therapy because of one self-removal of the drain, one obstruction of the drain, and two cases of intrapleural sclerosis. These four patients were excluded from the analysis of toxicity. The three treatment arms were well balanced for age, sex, and PS.

3.2. Treatment compliance and toxicity

Table 2 outlines the compliance with treatment. Fifty-one (50.0%) of the eligible patients completed intrapleural therapy and systemic chemotherapy as defined by the protocol. Forty-one (40.1%) of the eligible patients did not receive systemic chemotherapy because of disease progression. Two

Table 1 Patient Characteristics

Characteristic	BLM	OK-432	PE
All patients	36	34	35
Eligible patients	35	33	34
Age (years)			
Median	64	60.5	61
Range	44–75	31–73	39–75
Sex			
Male	24	21	24
Female	12	13	11
PS (ECOG)[a]			
0	2	4	2
1	30	27	28
2	4	3	5
≥10% weight loss within 6 m			
No	33	27	31
Yes	3	7	4
Histology			
Adenocarcinoma	29	32	32
Squamous cell	4	1	3
Large cell	1	1	0
Other	1	0	0
TNM (N factor)			
N0	14	14	14
N1	2	0	2
N2	16	13	11
N3	3	7	8
Stage			
IIIB	23	17	25
IV	12	17	10

[a] At the time of reexpansion of the affected lung.

patients (5.7%) in the BLM arm had pneumonitis induced by BLM and one of them had treatment-related death. One patient in the PE group did not receive systemic chemotherapy due to elevation of serum creatinine. Other reasons for noncompletion of the protocol treatment were two

Table 2 Treatment compliance

Variable	BLM	OK-432	PE
Eligible patients	35	33	34
No therapy	1	2	1
End of study protocol	18	19	14
Progressive disease	14	11	16
Toxicity	1	0	1
Death	1	0	0
Patient refusal	0	1	1
Insufficient drainage	0	0	1

patient refusals in each for the OK-432 and the PE arms, and one patient in the PE arm who could not receive sufficient drainage due to self-removal of the drain 48 h after intrapleural therapy.

Toxicities for intrapleural therapy in the three arms are listed in Table 3. Hematological toxic events were well tolerated in the three arms. Grade 4 nonhematological toxicity was not found in the three arms. Grade 2–3 chest pain occurred almost equally in the three arms. Grade 2–3 fever and nausea/vomiting occurred most frequently in the OK-432 arm (59.4%) and the PE arm (50.0%), respectively.

3.3. PPFS and OS

All eligible patients in the three arms were included in the survival analysis. PPFS and OS data are shown in Figs. 2 and 3, respectively. Median PPFS for the BLM arm was 20.9 weeks (95% confidence interval (CI), 4.7–25.9 weeks); for the OK-432 arm, 27.9 weeks (95% CI, 18.6–50.0 weeks); and for the PE arm, 18.4 weeks (95% CI, 4.4–41.4 weeks). The 4-week PPFS rate, which was the primary endpoint of this study, was 68.6% for the BLM arm (95% CI, 53.2–84.0%); 75.8% for the OK-432 arm (95% CI, 61.1–90.4%); and 70.6% for the PE arm (95% CI, 55.3–85.9%). The median survival time (MST) for the BLM arm was 32.1 weeks (95% CI, 21.6–37.9 weeks); 48.1 weeks for the OK-432 arm (95% CI, 26.7–58.4 weeks); and 45.7 weeks for the PE arm (95% CI, 34.4–57.1 weeks). The 48-week survival rate for the BLM arm was 29.9% (95% CI, 14.4–45.3%); 51.1% for the OK-432 arm (95% CI,

Table 3 Toxicity (JCOG grade) for Intrapleural Therapy

	BLM (n = 35)				OK-432 (n = 32)				PE (n = 34)			
	1	2	3	4	1	2	3	4	1	2	3	4
Leukocytes	3	3	0	1	1	0	1	0	8	3	2	1
Neutrophils	1	0	2	1	0	0	1	0	5	5	1	2
Hemoglobin	3	5	3	ND	3	6	1	ND	6	6	3	ND
Platelet	0	0	1	0	0	0	0	0	1	1	0	0
AST	8	0	0	0	15	2	0	0	6	0	0	0
ALT	11	0	0	0	14	7	0	0	10	2	0	0
Serum creatinine	1	0	0	0	0	0	0	0	4	1	0	0
Chest pain	10	5	4	0	15	8	1	0	13	6	1	0
Fever	12	13	0	0	6	18	1	0	9	7	2	0
Nausea/vomiting	7	3	0	ND	5	0	0	ND	10	13	4	ND

Abbreviation: ND, not defined.

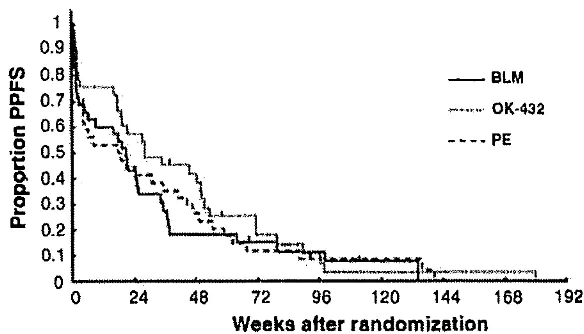


Fig. 2 Pleural progression-free survival (PPFS) in all eligible patients ($n = 102$).

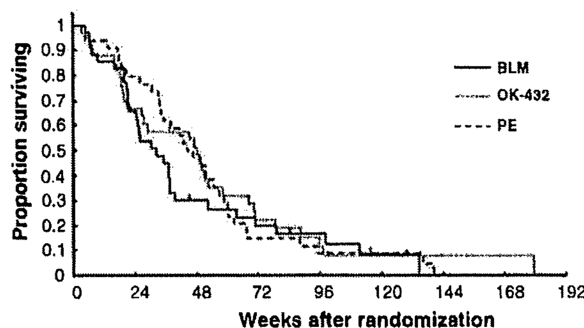


Fig. 3 Overall survival in all eligible patients ($n = 102$).

34.0–68.3%); and 47.1% for the PE arm (95% CI, 30.3–63.8%). Both the PPFS and OS for the OK-432 arm were superior to those for other two arms; however, the outcomes did not differ significantly between groups.

4. Discussion

To date, numerous chemical agents for treatment of MPE have been studied. These were antibiotics, antineoplastic agents, biological response modifiers (BRMs) and others that showed varied degrees of chemical sclerosis. Among them, BLM and talc are most frequently used for the management of MPE [5,7,17,18]. BLM is an antineoplastic antibiotic used in sclerotherapy with a success rate of 63–85% [7,8,18–21]. Talc applied as either slurry or poudrage is superior to other commonly used sclerosing agents with a success rate of 71–100% [5,7,22–24]. Because talc has not been available commercially in Japan and the use of talc was considered controversial at the beginning of this study because of severe complications, such as acute respiratory distress syndrome [25,26], we selected BLM as the sclerosing agent. A recent report demonstrated that the safety of talc pleurodesis and that acute respiratory distress syndrome can be avoided by using large-particle talc applied as thoracoscopic poudrage [27]. The thoracoscopic pleurodesis with talc is now considered to be the gold standard treatment for MPE [28,29].

OK-432 has been used as a BRM for gastric and lung cancer [9,10,30,31]. OK-432 has been reported to be effective in controlling MPE in two prospective randomized trials. One study reported a 73% success rate with OK-432 compared to 41% with mitomycin C treatment ($p = 0.03$) [11]. The other comparison found OK-432 70% effective compared to 46% in BLM subjects (statistical data not reported) [12]. OK-432 has been reported to induce various cytokines, such as tumor necrosis factor-α, interferon-γ, interleukin (IL)-1, IL-8 and IL-12 [32] and also to enhance cytotoxicity against tumor cells [33,34]. It is suggested that the main therapeutic effects of OK-432 for malignant effusion depend on increased expression of intercellular adhesion molecule-1 on tumor cells induced by interferon-γ [35].

Intrapleural combination chemotherapy is focused on achieving higher concentrations in the pleural cavity with less toxicity than systemic chemotherapy [36]. Two phase II studies with intrapleural cisplatin and cytarabine had success rates of 49% [2] and 73% [37]. Tohda et al. [13] reported that intrapleural instillation of cisplatin and etoposide for NSCLC with MPE resulted in a 46.2% overall response rate and the MST of 8 months was found to be improved, compared with previous reports for NSCLC with MPE of 3–6 months [11,18,38]. The reason for this was assumed to be that intrapleural combination chemotherapy of cisplatin and etoposide produced systemic as well as local effects. The overall response rates of intrapleural combination chemotherapy are variable and there are no prospective randomized studies compared modality of intrapleural combination chemotherapy with that of sclerotherapy.

There have been several special problems raised in the clinical trials for MPE, such as patient selection, response criteria, treatment procedures, short life expectancy, small sample sizes, and different endpoints [2–7,11,39]. To minimize the bias of patient selection, NSCLC patients with MPE who had received no prior therapy were entered into this study. Furthermore, justifiable and simplified response criteria and whether further treatment was required or not, as suggested by Ruckdeschel [18] and Rusch [40] were used and single intrapleural instillation of each agent was permitted to allow uniform estimation of responses. In many trials, successful pleurodesis was determined by assessing clinical and radiological findings. The positive response criteria have been defined generally as no pleural re-accumulation, 50% less effusion than that observed in the baseline radiograph taken immediately after the procedure, or no requirement for further thoracentesis. To determine the efficacy, we used the criterion that a decrease in effusion over one-quarter of the treated lung provides a stricter assessment of chemical pleurodesis that may relieve the symptoms of MPE. The position rotation after intrapleural instillation was recommended traditionally because it was thought to allow the agents to be distributed thoroughly throughout the entire pleural space. In contrast, studies using tetracycline and talc [41,42] demonstrated that rotation does not affect the overall intrapleural dispersion. It is unclear whether rotation is beneficial or not when applying the agents used in this study. Because a previous phase II study [13] showed that etoposide remains for a long period (β-phase half-life = 62.53 h) in intrapleural fluids, we applied the longer duration of clamping in the PE arm (72 h) than the other two arms (3 h) to provide enough exposure to the cancer cells. We found no major safety concerns such as excess pleural effusion as a result of the longer duration of clamping.

In this study, all three regimens were feasible. One treatment-related death occurred in the BLM arm 9 weeks after intrapleural instillation of BLM. Treatment compliance

rates for both intrapleural and systemic therapy was 50% (51 of the 102 eligible patients). This study lacks sufficient power to demonstrate differences between treatment arms; however, the OK-432 arm seemed to demonstrate modest benefit compared with the other two arms in terms of PPFS. It is assumed that the favorable efficacy in the OK-432 arm suggests that OK-432 has clinically meaningful activity for controlling MPE in NSCLC patients. NSCLC patients with MPE have been treated as patients with stage IV disease even when without metastasis, and systemic chemotherapy should be recommended when they have a good PS [43]. We prescribed systemic PE chemotherapy regimens, which were considered one of the standard regimens at the beginning of the study, following successful pleurodesis. However, we expect that platinum-based systemic combination chemotherapy regimens with several active new chemotherapeutic agents such as taxanes (paclitaxel and docetaxel), vinorelbine, gemcitabine and irrinotecan, which are the current standard treatment options for patients with advanced NSCLC, should enhance the survival benefit more than PE regimens.

This is the first fully reported randomized study that has evaluated the efficacy of intrapleural therapy for previously untreated patients with NSCLC and compliance with sequential systemic chemotherapy. As the results of this study demonstrate that intrapleural therapy with OK-432 shows a tendency to be more effective than BLM or PE in the management of MPE in NSCLC, in terms of PPFS, further studies are needed to compare OK-432 with talc.

Conflict of interest

None declared.

Acknowledgements

We are indebted to Ms. M. Imai and Dr. M. Niimi for data management and to Dr. N. Ishizuka for statistical analysis. We thank all of the investigators who contributed to study development and patient enrollment.

Supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan.

References

[1] Figlin R, Mendoza E, Piantadosi S, Rusch V. Intrapleural chemotherapy without pleurodesis for malignant pleural effusions. LCSG Trial 861. Chest 1994;106:363S–6S.

[2] Rusch VW, Figlin R, Godwin D, Piantadosi S. Intrapleural cisplatin and cytarabine in the management of malignant pleural effusions: a Lung Cancer Study Group trial. J Clin Oncol 1991;9:313–9.

[3] Windsor PG, Como JA, Windsor KS. Sclerotherapy for malignant pleural effusions: alternatives to tetracycline. South Med J 1994;87:709–14.

[4] Walker-Renard PB, Vaughan LM, Sahn SA. Chemical pleurodesis for malignant pleural effusions. Ann Intern Med 1994;120:56–64.

[5] Grossi F, Pennucci MC, Tixi L, et al. Management of malignant pleural effusions. Drugs 1998;55:47–58.

[6] Ruckdeschel JC. Management of malignant pleural effusions. Semin Oncol 1995;22:58–63.

[7] Zimmer PW, Hill M, Casey K, et al. Prospective randomized trial of talc slurry vs bleomycin in pleurodesis for symptomatic malignant pleural effusions. Chest 1997;112:430–4.

[8] Sartori S, Tassinari D, Ceccotti P, et al. Prospective randomized trial of intrapleural bleomycin versus interferon alfa-2b via ultrasound-guided small-bore chest tube in the palliative treatment of malignant pleural effusions. J Clin Oncol 2004;22:1228–33.

[9] Kimura I, Ohnoshi T, Yasuhara S, et al. Immunochemotherapy in human lung cancer using the streptococcal agent OK-432. Cancer 1976;37:2201–3.

[10] Sakamoto J, Teramukai S, Watanabe Y, et al. Meta-analysis of adjuvant immunochemotherapy using OK-432 in patients with resected non-small-cell lung cancer. J Immunother 2001;24:250–6.

[11] Luh KT, Yang PC, Kuo SH, et al. Comparison of OK-432 and mitomycin C pleurodesis for malignant pleural effusion caused by lung cancer. A randomized trial. Cancer 1992;69:674–9.

[12] Saka H, Shimokata K, Watanabe A, Saito H, Minami H, Sakai S. Randomized comparison of OK-432 and Bleomycin in intrapleural therapy for malignant effusions. Proc Am Soc Clin Oncol 1994;450a [Abstr 1555].

[13] Tohda Y, Iwanaga T, Takada M, et al. Intrapleural administration of cisplatin and etoposide to treat malignant pleural effusions in patients with non-small cell lung cancer. Chemotherapy 1999;45:197–204.

[14] Tobinai K, Kohno A, Shimada Y, et al. Toxicity grading criteria of the Japan Clinical Oncology Group. The Clinical Trial Review Committee of the Japan Clinical Oncology Group. Jpn J Clin Oncol 1993;23:250–7.

[15] Simon R, Wittes RE, Ellenberg SS. Randomized phase II clinical trials. Cancer Treat Rep 1985;69:1375–81.

[16] Armitage P, Berry G. Survival analysis. In: Statistical Methods in Medical Research. Oxford, United Kingdom: Blackwell Scientific Publications; 1994.

[17] Ostrowski MJ. Intracavitary therapy with bleomycin for the treatment of malignant pleural effusions. J Surg Oncol Suppl 1989;1:7–13.

[18] Ruckdeschel JC, Moores D, Lee JY, et al. Intrapleural therapy for malignant pleural effusions. A randomized comparison of bleomycin and tetracycline. Chest 1991;100:1528–35.

[19] Paladine W, Cunningham TJ, Sponzo R, et al. Intracavitary bleomycin in the management of malignant effusions. Cancer 1976;38:1903–8.

[20] Bitran JD, Brown C, Desser RK, et al. Intracavitary bleomycin for the control of malignant effusions. J Surg Oncol 1981;16:273–7.

[21] Ostrowski MJ, Priestman TJ, Houston RF, Martin WM. A randomized trial of intracavitary bleomycin and *Corynebacterium parvum* in the control of malignant pleural effusions. Radiother Oncol 1989;14:19–26.

[22] Hamed H, Fentiman IS, Chaudary MA, Rubens RD. Comparison of intracavitary bleomycin and talc for control of pleural effusions secondary to carcinoma of the breast. Br J Surg 1989;76:1266–7.

[23] Hartman DL, Gaither JM, Kesler KA, et al. Comparison of insufflated talc under thoracoscopic guidance with standard tetracycline and bleomycin pleurodesis for control of malignant pleural effusions. J Thorac Cardiovasc Surg 1993;105:743–7 [Discussion747–8].

[24] Dresler CM, Olak J, Herndon II JE, et al. Phase III intergroup study of talc poudrage vs talc slurry sclerosis for malignant pleural effusion. Chest 2005;127:909–15.

[25] Rinaldo JE, Owens GR, Rogers RM. Adult respiratory distress syndrome following intrapleural instillation of talc. J Thorac Cardiovasc Surg 1983;85:523–6.

[26] Bouchama A, Chastre J, Gaudichet A, et al. Acute pneumonitis with bilateral pleural effusion after talc pleurodesis. Chest 1984;86:795–7.
[27] Janssen JP, Collier G, Astoul P, et al. Safety of pleurodesis with talc poudrage in malignant pleural effusion: a prospective cohort study. Lancet 2007;369:1535–9.
[28] Shaw P, Agarwal R. Pleurodesis for malignant pleural effusions. Cochrane Database Syst Rev (database online) 2004.
[29] Aelony Y. Talc pleurodesis and acute respiratory distress syndrome. Lancet 2007;369:1494–6.
[30] Yasue M, Murakami M, Nakazato H, et al. A controlled study of maintenance chemoimmunotherapy vs immunotherapy alone immediately following palliative gastrectomy and induction chemoimmunotherapy for advanced gastric cancer. Tokai cooperative study group for adjuvant chemoimmunotherapy of stomach cancer. Cancer Chemother Pharmacol 1981;7:5–10.
[31] Watanabe Y, Iwa T. Clinical value of immunotherapy with the streptococcal preparation OK-432 in non-small cell lung cancer. J Biol Response Mod 1987;6:169–80.
[32] Katano M, Morisaki T. The past, the present and future of the OK-432 therapy for patients with malignant effusions. Anticancer Res 1998;18:3917–25.
[33] Uchida A, Micksche M. Lysis of fresh human tumor cells by autologous peripheral blood lymphocytes and pleural effusion lymphocytes activated by OK432. J Natl Cancer Inst 1983;71:673–80.
[34] Wei Y, Zhao X, Kariya Y, et al. Induction of autologous tumor killing by heat treatment of fresh human tumor cells: involvement of gamma delta T cells and heat shock protein 70. Cancer Res 1996;56:1104–10.
[35] Kitsuki H, Katano M, Ikubo A, et al. Induction of inflammatory cytokines in effusion cavity by OK-432 injection therapy for patients with malignant effusion: role of interferon-gamma in enhancement of surface expression of ICAM-1 on tumor cells in vivo. Clin Immunol Immunopathol 1996;78:283–90.
[36] Markman M. Intracavitary chemotherapy. Curr Probl Cancer 1986;10:401–37.
[37] Aitini E, Cavazzini G, Pasquini E, et al. Treatment of primary or metastatic pleural effusion with intracavitary cytosine arabinoside and cisplatin. A phase II study. Acta Oncol 1994;33:191–4.
[38] Sugiura S, Ando Y, Minami H, et al. Prognostic value of pleural effusion in patients with non-small cell lung cancer. Clin Cancer Res 1997;3:47–50.
[39] Miles DW, Knight RK. Diagnosis and management of malignant pleural effusion. Cancer Treat Rev 1993;19:151–68.
[40] Rusch VW. The optimal treatment of malignant pleural effusions. A continuing dilemma. Chest 1991;100:1483–4.
[41] Dryzer SR, Allen ML, Strange C, et al. A comparison of rotation and nonrotation in tetracycline pleurodesis. Chest 1993;104:1763–6.
[42] Mager HJ, Maesen B, Verzijlbergen F, et al. Distribution of talc suspension during treatment of malignant pleural effusion with talc pleurodesis. Lung Cancer 2002;36:77–81.
[43] Pfister DG, Johnson DH, Azzoli CG, et al. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. J Clin Oncol 2004;22:330–53.

Drug Metab. Pharmacokinet. 22 (6): *SNP50* (456)-*SNP55* (461) (2007).

SNP Communication

*Genetic Variations and Frequencies of Major Haplotypes in SLCO1B1 Encoding the Transporter OATP1B1 in Japanese Subjects: SLCO1B1*17 is More Prevalent Than *15*

Su-Ryang KIM[1], Yoshiro SAITO[1,2,*], Kimie SAI[1,3], Kouichi KUROSE[1,4], Keiko MAEKAWA[1,2], Nahoko KANIWA[1,4], Shogo OZAWA[1,5,†], Naoyuki KAMATANI[6], Kuniaki SHIRAO[7], Noboru YAMAMOTO[7], Tetsuya HAMAGUCHI[7], Hideo KUNITOH[7], Yuichiro OHE[7], Yasuhide YAMADA[7], Tomohide TAMURA[7], Teruhiko YOSHIDA[8], Hironobu MINAMI[9,‡], Atsushi OHTSU[10], Nagahiro SAIJO[11] and Jun-ichi SAWADA[1,2]

[1]Project Team for Pharmacogenetics, [2]Division of Biochemistry and Immunochemistry, [3]Division of Biosignaling, [4]Division of Medicinal Safety Science, [5]Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan
[6]Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan
[7]Division of Internal Medicine, National Cancer Center Hospital,
[8]Genomics Division, National Cancer Center Research Institute, National Cancer Center, Tokyo, Japan
[9]Division of Oncology/Hematology, [10]Division of GI Oncology/Digestive Endoscopy,
[11]Deputy Director, National Cancer Center Hospital East, National Cancer Center, Chiba, Japan

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: A liver-specific transporter organic anion transporting polypeptide 1B1 (OATP1B1, also known as OATP-C) is encoded by *SLCO1B1* and mediates uptake of various endogenous and exogenous compounds from blood into hepatocytes. In this study, 15 *SLCO1B1* exons (including non-coding exon 1) and their flanking introns were comprehensively screened for genetic variations in 177 Japanese subjects. Sixty-two genetic variations, including 28 novel ones, were found: 7 in the 5′-flanking region, 1 in the 5′-untranslated region (UTR), 13 in the coding exons (9 nonsynonymous and 4 synonymous variations), 5 in the 3′-UTR, and 36 in the introns. Five novel nonsynonymous variations, 311T>A (Met104Lys), 509T>C (Met170Thr), 601A>G (Lys201Glu), 1553C>T (Ser518Leu), and 1738C>T (Arg580Stop), were found as heterozygotes. The allele frequencies were 0.008 for 1738C>T (Arg580Stop) and 0.003 for the four other variations. Arg580Stop having a stop codon at codon 580 results in loss of half of transmembrane domain (TMD) 11, TMD12, and a cytoplasmic tail, which might affect transport activity. In addition, novel variations, IVS12-1G>T at the splice acceptor site and -3A>C in the Kozak motif, were detected at 0.003 and 0.014 frequencies, respectively. Haplotype analysis using -11187G>A, -3A>C, IVS12-1G>T and 9 nonsynonymous variations revealed that the haplotype frequencies for **1b*, **5*, **15*, and **17* were 0.469, 0.000 (not detected), 0.037, and 0.133, respectively. These data would provide fundamental and useful information for pharmacogenetic studies on OATP1B1-transported drugs in Japanese.

Key words: ***SLCO1B1*; direct sequencing; novel genetic variation; amino acid change**

Received; July 18, 2007, Accepted; September 4, 2007
**To whom correspondence should be addressed:* Yoshiro SAITO, Ph.D., *Division of Biochemistry and Immunochemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel. +81-3-5717-3831, Fax. +81-3-5717-3832, E-mail: yoshiro@nihs.go.jp*
†Present address: Department of Pharmacodynamics and Molecular Genetics, Faculty of Pharmaceutical Sciences, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba-cho, Shiwa-gun, Iwate 028-3694, Japan.
‡Present address: Medical Oncology, Department of Medicine, Kobe University Hospital and Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan.

Introduction

Organic anion transporting polypeptide 1B1 (OATP1B1, also known as OATP-C, OATP2 and LST-1) is a liver-specific transporter expressed on the sinusoidal membrane and mediates uptake of various endogenous and exogenous compounds from blood into hepatocytes.[1,2] Exogenous compounds include several 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (such as pravastatin), an active metabolite of irinotecan SN-38, methotrexate, and rifampicin; endogenous substrates include bilirubin and bilirubin glucuronide, cholate, leukotriene C_4, and estradiol-17β-glucuronide.[1,2]

OATP1B1 protein (691 amino acid residues) is encoded by *SLCO1B1*, which consists of 15 exons (including non-coding exon 1) and spans approximately 109 kb on chromosome 12p12.2-p12.1. Similar to other OATP family members, this transporter is predicted to have 12 transmembrane domains (TMDs).[1,3]

Several genetic polymorphisms and haplotypes with functional significance are already known in *SLCO1B1*. In Japanese, two haplotypes with nonsynonymous variations **1b* and **15* have been frequently reported. The *SLCO1B1*1b* haplotype with 388A>G (Asn130Asp) has been shown to have no altered transport activity from *in vitro* expression systems.[2,4-7] Recently, however, an *in vivo* study has suggested that the area under the concentration-time curve (AUC) of pravastatin is significantly lower in **1b/*1b* subjects than in **1a/*1a* subjects, suggesting increased transport activity possibly through increased protein expression.[8] Another major haplotype, *SLCO1B1*15* harboring both 388A>G (Asn130Asp) and 521T>C (Val174Ala), has been reported to show impaired plasma membrane expression[6] and reduced transport activity *in vitro*,[6,7] probably due to the Val174Ala substitution.[2,4,6] The association of the **15* haplotype with significant increases in AUC was reported for pravastatin,[2,9] and irinotecan and SN-38.[10] The haplotype frequencies of **1b*, **5* (with 521T>C, Val174Ala), and **15* were reported to be 0.46-0.54, 0.00-0.01, and 0.10-0.15, respectively, in Japanese.[5,11]

Recently, the *SLCO1B1*17* haplotype having 388A>G (Asn130Asp), 521T>C (Val174Ala), and -11187G>A was also shown to increase the AUC of pravastatin[9] and likely reduces the pravastatin efficacy on cholesterol synthesis,[12] although the effect of -11187G>A on transcriptional activity has not been clarified *in vitro*. The frequency of **17* has not, however, been reported in Japanese.

In this study, all 15 exons and their surrounding introns were resequenced for comprehensive screening of genetic variations in *SLCO1B1*. Sequence analysis detected 62 variations including 5 novel nonsynonymous ones from 177 Japanese subjects. Haplotype frequencies of **1b*, **5*, **15*, and **17* were also estimated.

Materials and Methods

Human genomic DNA samples: One hundred seventy-seven Japanese cancer patients administered irinotecan participated in this study and provided written informed consent. The ethical review boards of the National Cancer Center and the National Institute of Health Sciences approved this study. Whole blood was collected from the patients prior to the administration of irinotecan, and genomic DNA was extracted from blood leukocytes by standard methods.

PCR conditions for DNA sequencing and haplotype analysis: First, two sets of multiplex PCR were performed to amplify all 15 exons of *SLCO1B1* from 100 ng of genomic DNA using 1.25 units of *Z-Taq* (Takara Bio. Inc., Shiga, Japan) with 0.2 μM each of the mixed primers (Mix 1 and Mix 2) designed in the intronic regions as listed in **Table 1** (1st PCR). Mix 1 contained primers for amplifying exons 1 and 2, and 12 to 14, and Mix 2 contained primers for exons 3 to 7, 8 to 11, and 15. The first PCR conditions consisted of 30 cycles of 98 °C for 5 sec, 55°C for 10 sec, and 72°C for 190 sec. Next, each exon was amplified separately by *Ex-Taq* (0.625 units, Takara Bio. Inc.) with appropriate primers (0.5 μM) designed in the introns (**Table 1**, 2nd PCR). The conditions for the second round PCR were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. For amplification of exons 10 and 13, PCR was carried out under the following conditions: 94°C for 5 min followed by 33 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 30 sec, and then a final extension at 72°C for 7 min. Following PCR, the products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in **Table 1** (Sequencing). Excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All variations were confirmed by sequence analysis of PCR products generated by new amplification of the original genomic DNA templates.

As of July 18, 2007, the novel variations reported here are not found in the database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB Database (http://www.pharmgkb.org/).

This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, and the Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare.

Table 1. Primer sequences used in this study

		Amplified or sequenced region	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified region[a]	Length (bp)
1st PCR	Mix 1	Exons 1 and 2	ACTCTGGGGCTAAAACCTATTGGAC	CTGCTTGCCATAACATCTTGAGGGT	14041049_14055679	14,631
		Exons 12 to 14	CTAGGGCTTTTATTGATAGGCAGGT	AAACTTCCAGACTGTCTTCTACCAT	14127745_14137752	10,008
	Mix 2	Exons 3 to 7	TTTGTTGAGAAGAGACTGTTAGGCA	GGAAAATGGATGAAGAAGCACTGGA	14083429_14092020	8,592
		Exons 8 to 11	AAGGACAGCACCAAGCAATGAAGGA	CATTCAACTCAGCAATCCCACTACC	14106917_14119592	12,676
		Exon 15	CTGAGGAGAACTGTAATTTGATGTC	TTTCCAGAGGCAAGCATTTACAACT	14148708_14152868	4,161
2nd PCR		Exon 1	TAACAGGCATAATCTTTGGTCT	AAGGGCTCAGAATGTAAGCG	14041915_14043281	1,367
		Exon 2	TCCTTAGGCTAGAATTTGTGT	CAAAGTGAGTCTCAAGACATT	14053227_14053950	724
		Exon 3	TGGCTGAGTAGTAGTACCTG	ATCCTCACTATCAACATTTTCA	14084394_14084961	568
		Exon 4	TGAGTGGTCTAATGTAGGTGA	AGGTGTAAGTGTTGAGGTCTT	14086324_14086946	623
		Exon 5	ATCTTTCTTGCTGGACACTTC	TATTAAGGAATTTGTTACAGGG	14088485_14089158	674
		Exons 6 and 7	CATAAGAATGGACTAATACACC	GGGAGACATTTTACATTTGGTT	14090310_14091202	893
		Exon 8	TTCCTAGACAGTATCTGTTGC	CTTCCACTTGTTATGTGCTCA	14108658_14109289	632
		Exon 9	AGTTACAAAACAGCACTTACG	TCAGGAACTCATCTAAAATAAG	14112156_14112798	643
		Exon 10	CAGGGGTTAAAACCTAGATGA	ATCCATGTATTTCTCTAAGCC	14114169_14114904	736
		Exon 11	TGGCAAAGATGGAGAGCGTA	AGTCAAATGAGGTGCTTCTTA	14117563_14118256	694
		Exon 12	TTGTCCAAAAGAGTATGTGCT	CAGCCTTGAGAGTTCATAGT	14128668_14129375	708
		Exon 13	TTGACCCAGCAATCCAACTAT	CCTTTTTTTTTCATCATACCTAGT[b]	14133788_14134290	503
		Exon 14	ATATTAACCAACATAACTTCCA	CCTTGAATCACAGTTTCTTCG	14136297_14136980	684
		Exon 15	GATGGCTTAACAGGGCTTGA	TGCGGCAAATGATCTAGGAA	14150619_14151844	1,226
Sequencing		Exon 1	TAACAGGCATAATCTTTGGTCT	AAGGGCTCAGAATGTAAGCG		
			TATGTGAGAGAAGGGTCTGTA	CTACAGGTTACATTGGCATTT		
			AAATGCCAATGTAACCTGTAG	CTGAAATAAAGTACAGACCCT		
		Exon 2	TCCTTAGGCTAGAATTTGTGT	CAAAGTGAGTCTCAAGACATT		
		Exon 3	TGGCTGAGTAGTAGTACCTG	ATCCTCACTATCAACATTTTCA		
		Exon 4	TGAGTGGTCTAATGTAGGTGA	AGGTGTAAGTGTTGAGGTCTT		
		Exon 5	ATCTTTCTTGCTGGACACTTC	TATTAAGGAATTTGTTACAGGG		
		Exon 6	TTAAGAGTTTACAAGTAGTTAAA	AAGCAATTTTACTAGATGCCAA		
		Exon 7	CTTCTTTGTATTTAGGTAATGTA	ATAGTATAAATAGGAGCTGGAT		
		Exon 8	TTCCTAGACAGTATCTGTTGC	CTTCCACTTGTTATGTGCTCA		
		Exon 9	AGTTACAAAACAGCACTTACG	TCAGGAACTCATCTAAAATAAG		
		Exon 10	TTGATAGGTGCAGCAAACCAC	GGAATAAAAGAATGTGTTTGAG		
		Exon 11	TCTTTTTGATATATGTCTATCAT	AGTCAAATGAGGTGCTTCTTA		
		Exon 12	TTGTCCAAAAGAGTATGTGCT	CAGCCTTGAGAGTTCATAGT		
		Exon 13	GTTCTAACCACTTCCTCATAG	CCTTTTTTTTTCATCATACCTAGT		
		Exon 14	TCCTTTTTACCATTCAGGCTTA	ACTAAAATGAGATACGAGATTG		
		Exon 15	GATGGCTTAACAGGGCTTGA	TGCGGCAAATGATCTAGGAA		
			CACATCTTTTATGGTGGAAGT	AGGCTTATTTATACTTCCACC		

[a]The reference sequence is NT_009714.16.
[b]Mismatched nucleotides at the 5′ end are underlined.

Furthermore, rare SNPs found in single patients as heterozygotes were confirmed by sequencing the PCR fragments produced by the amplification with a high fidelity DNA polymerase KOD-Plus- (TOYOBO, Tokyo, Japan).

Hardy-Weinberg equilibrium was analyzed by SNPAlyze version 3.1 (Dynacom Co., Yokohama, Japan). Estimation of *SLCO1B1* haplotypes was performed by an expectation-maximization based program, LDSUPPORT software.[13)]

Results and Discussion

Sequence analysis from 177 Japanese subjects resulted in the identification of 62 genetic variations, including 28 novel ones (**Table 2**). Of these variations, 7 were located in the 5′-flanking region, 1 in the 5′-untranslated region (UTR), 13 in the coding exons (9 nonsynonymous and 4 synonymous variations), 5 in the 3′-UTR, and 36 in the introns. All detected variations were in Hardy-Weinberg equilibrium ($p > 0.05$).

Of the 9 nonsynonymous variations, 5 variations were novel: 311T>A (Met104Lys), 509T>C (Met170Thr), 601A>G (Lys201Glu), 1553C>T (Ser518Leu), and 1738C>T (Arg580Stop). All of these variations were found as heterozygotes with frequencies of 0.008 for 1738C>T (Arg580Stop) and 0.003 for the four other variations. Arg580, residing in TMD11, is conserved among human, rat and mouse OATP families.[1)] The change from arginine residue to the immature termination codon leads to loss of this conserved amino acid

Table 2. Summary of *SLCO1B1* variations detected in this study

SNP ID			Reference	Location	Position		Nucleotide change	Amino acid change	Allele frequency (n=354)
This Study	dbSNP (NCBI)	JSNP			NT_009714.16	From the translational initiation site or from the end of the nearest exon			
MPJ6_SB1_001[a]				5'-flanking	14042128	-11355 (-1078)[b]	ccaatactctcaA/Gtaattaaccaag		0.003
MPJ6_SB1_002	rs4149015	ssj0003132	9, 15	5'-flanking	14042296	-11187 (-910)[b]	tatgtgtatacaG/Agtaaaagtgtgt		0.153
MPJ6_SB1_003[a]				5'-flanking	14042494	-10989 (-712)[b]	atctctactcaaG/Aaaaacttttaac		0.076
MPJ6_SB1_004[a]				5'-flanking	14042530	-10953 (-676)[b]	cttcctttctccA/Tcaagtcaagtca		0.003
MPJ6_SB1_005	rs11835045			5'-flanking	14042793	-10690 (-413)[b]	atttgtcttaaaT/Ctatttcctattt		0.076
MPJ6_SB1_006[a]				5'-flanking	14042860	-10623 (-346)[b]	ttaaagaaaaaaA/-tcttatgccacc		0.003
MPJ6_SB1_007[a]				5'-flanking	14043018	-10465 (-188)[b]	aactaggttttaT/Catgtttgactag		0.003
MPJ6_SB1_008[a]				Intron 1	14043209	IVS1+65	ttcacggaagaaG/Catttigatggtc		0.014
MPJ6_SB1_009	rs2010668	ssj0003141		Intron 1	14053267	IVS1-155	tcctacttttgtG/Ttccagcattgac		0.113
MPJ6_SB1_010[a]				5'-UTR	14053480	-3	atctatatttcaA/Ctcatggaccaaa		0.014
MPJ6_SB1_011[a]				Intron 2	14053635	IVS2+69	tagaaaagcaagT/Ctgttaaaaagaa		0.003
MPJ6_SB1_012[a]				Intron 2	14053648	IVS2+82	tgttaaaaagaaC/Tattatgtttcaa		0.003
MPJ6_SB1_013[a]				Intron 2	14053734	IVS2+168	aaaccagtctttT/Caatctgattaag		0.008
MPJ6_SB1_014	rs4149021	ssj0003142	9	Intron 2	14053759	IVS2+193	tatttctttggcG/Aaaatttttgatg		0.153
MPJ6_SB1_015	rs12812795		9	Intron 2	14053769	IVS2+203	gcgaaatttttgA/Ttgcttaatagtt		0.003
MPJ6_SB1_016[a]				Intron 2	14053807	IVS2+241	aatttagaaataT/Ctttgatagcttc		0.006
MPJ6_SB1_017	rs12303784			Intron 2	14053814	IVS2+248	aaatatttigatA/Ggcttctctttgg		0.003
MPJ6_SB1_018[a]				Intron 2	14084429	IVS2-129	aaagggaaaactA/Gagtatggttttt		0.003
MPJ6_SB1_019[a]				Intron 2	14084478	IVS2-80	aaagaagaaagcT/Cattataattcca		0.008
MPJ6_SB1_020	rs2291073	JST-043317		Intron 3	14084788	IVS3+89	actggggtaaatT/Gtatctctcacag		0.271
MPJ6_SB1_021	rs2291074	JST-043318		Intron 3	14084923	IVS3+224	attctataatgcA/Gcaaagaatgatg		0.243
MPJ6_SB1_022[a]				Exon 4	14086569	311	gttgtttcattaT/Agggaattggagg	Met104Lys	0.003
MPJ6_SB1_023	rs4149036	ssj0003160		Intron 4	14086714	IVS4+97	ataggcagttacC/Atttgagaagat		0.427
MPJ6_SB1_024[a]				Intron 4	14088523	IVS4-161	cactttacccaT/Ccacatctcttaa		0.017
MPJ6_SB1_025	rs2306283	JST-063865	4, 5, 9, 11, 15	Exon 5	14088712	388	gaaactaatatcA/Gattcatcagaaa	Asn130Asp	0.667
MPJ6_SB1_026	rs2306282	JST-063864	11	Exon 5	14088776	452	ttttatcactcaA/Gtagagcatcacc	Asn151Ser	0.034
MPJ6_SB1_027	rs4149044	ssj0003170	9	Intron 5	14088970	IVS5+165	cacagttcgcccA/Tttaacaacacag		0.427
MPJ6_SB1_028	rs4149045	ssj0003171	9	Intron 5	14088994	IVS5+189	ggtttaaactacG/Acgttttcacttc		0.429
MPJ6_SB1_029	rs4149046	ssj0003172	9	Intron 5	14088996	IVS5+191	tttaaactacgcG/Attttcacttcta		0.331
MPJ6_SB1_030	rs4149096	ssj0003230	9	Intron 5	14090372_14090377	IVS5-107_112	aaattacttgtaCTTGTA/-aattaaaaaaaa		0.427
MPJ6_SB1_031[a]				Intron 5	14090469	IVS5-15	aaatgaaacactC/Gtcttatctacat		0.003
MPJ6_SB1_032[a]				Exon 6	14090511	509	ctgggtcatacaT/Cgtggatatatgt	Met170Thr	0.003
MPJ6_SB1_033	rs4149056	ssj0003182	4, 5, 9, 11, 15	Exon 6	14090523	521	tgtggatatatgT/Cgttcatgggtaa	Val174Ala	0.175
MPJ6_SB1_034	rs4149057	ssj0003183	9, 11, 15	Exon 6	14090573	571	cccatagtaccaT/Ctggggctttctt	Leu191Leu	0.333
MPJ6_SB1_035[a]				Exon 6	14090578	576	agtaccattgggG/Actttcttacatt	Gly192Gly	0.003
MPJ6_SB1_036	rs2291075	JST-043319	9, 11, 15	Exon 6	14090599	597	cattgatgatttC/Tgctaaagaagga	Phe199Phe	0.427
MPJ6_SB1_037[a]				Exon 6	14090603	601	gatgatttcgctA/Gaagaaggacatt	Lys201Glu	0.003
MPJ6_SB1_038	rs2291076	JST-043320	9	Intron 7	14090961	IVS7+33	gtaccatgataaC/Tgtctttctaagc		0.336
MPJ6_SB1_039			11	Exon 9	14112452	1007	tccttactaatcC/Gcctgtatgttat	Pro336Arg	0.006
MPJ6_SB1_040[a]				Intron 9	14114331	IVS9-68	ttgacatacattG/Ctgtttcatctat		0.003
MPJ6_SB1_041	rs4149099	JST-080069	9	Intron 10	14117669_14117670	IVS10-106_-107	tttatctacttt-/CTTttttccctcttt		0.647
MPJ6_SB1_042			11	Intron 10	14117728_14117730	IVS10-46_-48	cttctttgtcttTTT/-cttctctctctc		0.003
MPJ6_SB1_043	rs4149070	ssj0003204	9	Intron 11	14128857	IVS11-170	gaaagaaatccaC/Gaaaactatttta		0.280
MPJ6_SB1_044	rs4149071	ssj0003205	9	Intron 11	14128938	IVS11-89	agtttgaacaagT/Cgagacttcacta		0.280
MPJ6_SB1_045	rs4149100	ssj0003234	9	Intron 11	14128952	IVS11-75	agacttcactaaA/-tataatgcaatg		0.395
MPJ6_SB1_046	rs4149072	ssj0003206	9	Intron 11	14128959	IVS11-68	actaaatataatG/Acaatgtatttgc		0.280
MPJ6_SB1_047			11	Intron 11	14129015	IVS11-12	catattttatacA/Gcaacgcttaagg		0.014
MPJ6_SB1_048[a]				Exon 12	14129082	1553	acagaaattactC/Tagcccatttggg	Ser518Leu	0.003
MPJ6_SB1_049	rs987839			Intron 12	14133812	IVS12-396	tccaactattggG/Atatctaccaaaa		0.316
MPJ6_SB1_050[a]				Intron 12	14134097	IVS12-111	ggggccattcaaC/Ttgtgagcttaat		0.020
MPJ6_SB1_051[a]				Intron 12	14134207	IVS12-1	tgtcttgtttcaG/Taattgttcaacc		0.003
MPJ6_SB1_052[a]				Exon 13	14134263	1738	tcaatggttataC/Tgagcactaggta	Arg580Stop	0.008
MPJ6_SB1_053	rs4149080	ssj0003214	9	Intron 13	14136533	IVS13-97	ctccaaattttttG/Caacttttattta		0.395
MPJ6_SB1_054	rs11045875		11	Intron 14	14136797	IVS14+50	gactatattaatT/Gcctaaaaaatat		0.011
MPJ6_SB1_055[a]				Intron 14	14150655	IVS14-232	tatatttttctcG/Atttatgaagaa		0.006
MPJ6_SB1_056[a]				Intron 14	14150656	IVS14-231	atattttctcgT/Ctttatgaagaag		0.251
MPJ6_SB1_057[a]				Exon 15	14151004	1983	tgcatcagaaaaT/Cggaagtgtcatg	Asn661Asn	0.006
MPJ6_SB1_058[a]				3'-UTR	14151137	2116 (*40)[c]	tgtgtttccaaaC/Gagcattgcattg		0.011
MPJ6_SB1_059	rs4149085	ssj0003219		3'-UTR	14151264	2243 (*167)[c]	acaaactgtaggT/Cagaaaaaatgag		0.251
MPJ6_SB1_060	rs4149086	ssj0003220		3'-UTR	14151425	2404 (*328)[c]	aaacaaatgagtA/Gtcatacaggtag		0.025
MPJ6_SB1_061	rs4149087	ssj0003221	15	3''-UTR	14151536	2515 (*439)[c]	gaactataatacG/Taaggcctgaagt		0.333
MPJ6_SB1_062	rs4149088	ssj0003222		3'-UTR	14151560	2539 (*463)[c]	tctagcttggatG/Atatgctacaata		0.333

[a]Novel variations detected in this study.
[b]Intron 1 is skipped for counting.
[c]Positions are shown as * and bases from the translational termination codon TAA.

along with the subsequent half of TMD11, TMD12 and the cytoplasmic tail,[1] which very likely affects transport activity. Other variations 311T>A (Met104Lys), 509T>C (Met170Thr), 601A>G (Lys201Glu), and 1553C>T (Ser518Leu) are located in TMD3, TMD4, the short cytoplasmic loop between TMD4 and TMD5, and the large extracellular loop between TMD9 and TMD10, respectively.[1] Using the PolyPhen program (http://genetics.bwh.harvard.edu/pph/) to predict the functional effects of the four amino acid substitutions, three substitutions, Met104Lys, Met170Thr and Ser518Leu, were expected to alter the protein function based on the PSIC (position-specific independent count) score differences derived from multiple alignments. The functional significance of these 5 novel nonsynonymous variations should be clarified in the future. In addition, a novel variation at the splice acceptor site, IVS12-1 G>T, was detected at a 0.003 frequency. This variation might cause aberrant splicing of *SLCO1B1* pre-mRNA and thus influence the expression level of active protein. Furthermore, -3A>C might reduce translational efficiency since this purine-to-pyrimidine alteration results in a deviation from the Kozak motif, where the purine nucleotide at position -3 from the translational initiation codon is important.[14]

Four known variations, 388A>G (Asn130Asp), 452A>G (Asn151Ser), 521T>C (Val174Ala), and 1007C>G (Pro336Arg), were detected at 0.667, 0.034, 0.175, and 0.006 frequencies, respectively, which are similar to the Japanese data reported previously.[5,11] The allele frequencies of 521T>C (Val174Ala) in Japanese (0.11–0.18) are comparable to those in other Asian populations (0.04–0.25) and Caucasians (0.14–0.22), but higher than that in African-Americans (0.02).[10,15,16] The frequencies of 388A>G (Asn130Asp) in Japanese (0.63–0.67) are also similar to those in other Asians (0.57–0.88) and African-Americans (0.75), but higher than those in Caucasians (0.30–0.51).[10,15,16] Variations 452A>G (Asn151Ser) and 1007C>G (Pro336Arg) have not been reported in other ethnic populations. Analysis of these four known variations with PolyPhen program showed that only Val174Ala was expected to alter protein function, which is consistent with the previous functional analysis.[2,4,6] Variations 1454G>T (Cys485Phe) and 1628T>G (Leu543Trp) previously reported in Japanese were not detected in this study.[11,17] Hepatocyte nuclear factor 1α is known to transactivate *SLCO1B1* through binding to the promoter region (from -10432 to -10420 from the translational start codon);[18] however, no variation was found in this region.

Using -11187G>A, -3A>C, IVS12-1G>T and 9 nonsynonymous variations, diplotype configuration was estimated for each subject. The configuration was estimated with >0.99 probabilities for all but four subjects. The predicted haplotype frequencies for **1b* [harboring 388A>G (Asn130Asp)], **5* [harboring 521T>C (Val174Ala)], **15* [harboring 388A>G (Asn130Asp) and 521T>C (Val174Ala)] and **17* [harboring -11187G>A, 388A>G (Asn130Asp), and 521T>C (Val174Ala)] were 0.469, 0.000 (not detected), 0.037 and 0.133, respectively. The haplotype frequencies for **1b* and **5* are similar to those in the previous studies in Japanese.[5,11] The **17* frequency is higher than those in Chinese (0.085), Finnish Caucasians (0.069), Malay (0.029) and Indians (0.009).[15,16] It should be noted that 76% (n=47 alleles) of 521T>C (Val174Ala)-bearing haplotypes were assigned as **17*, and 21% (n=13) of them as **15*. The remaining two (3%) was estimated to exist with 1007C>G (Pro336Arg) and **17* variations [-11187G>A, 388A>G (Asn130Asp), and 521T>C (Val174Ala)] on the same chromosomes. The **17* ratio in 521T>C (Val174Ala)-bearing haplotypes is similar to that in Chinese (65%), but higher than those in Finnish Caucasians (34%), Malay (26%) and Indians (14%).[15,16] Variation 452A>G (Asn151Ser, n=12 alleles) or 1738C>T (Arg580Stop, n=3) were predicted to be on the **1a* background (no other variation).

In conclusion, 62 genetic variations were identified, including 28 novel ones, in *SLCO1B1*. One novel nonsynonymous variation results in a truncated protein and four novel nonsynonymous variations result in amino acids substitutions. In addition, novel variations IVS12-1 G>T at the splice acceptor site and -3A>C in the Kozak motif were detected. Approximately 76% of 521T>C (Val174Ala)-bearing haplotypes were assigned as **17* and the majority of the remaining haplotypes were **15*. This information would be useful for pharmacogenetic studies to investigate the associations of *SLCO1B1* variations with interindividual differences in drug disposition.

Acknowledgments: The authors thank Ms. Chie Sudo for her secretarial assistance.

References

1) Hagenbuch, B. and Meier, P. J.: The superfamily of organic anion transporting polypeptides. *Biochim. Biophys. Acta.*, **1609**: 1–18 (2003).
2) Kivisto, K. T. and Niemi, M.: Influence of drug transporter polymorphisms on pravastatin pharmacokinetics in humans. *Pharm. Res.*, **24**: 239–247 (2007).
3) Tamai, I., Nezu, J., Uchino, H., Sai, Y., Oku, A., Shimane, M. and Tsuji, A.: Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem. Biophys. Res. Commun.*, **273**: 251–260 (2000).
4) Tirona, R. G., Leake, B. F., Merino, G. and Kim, R. B.: Polymorphisms in OATP-C: Identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J. Biol.*

Chem., **276**: 35669-35675 (2001).

5) Nozawa, T., Nakajima, M., Tamai, I., Noda, K., Nezu, J., Sai, Y., Tsuji, A. and Yokoi, T.: Genetic polymorphisms of human organic anion transporters OATP-C (SLC21A6) and OATP-B (SLC21A9): allele frequencies in the Japanese population and functional analysis. *J. Pharmacol. Exp. Ther.*, **302**: 804-813 (2002).
6) Kameyama, Y., Yamashita, K., Kobayashi, K., Hosokawa, M. and Chiba, K.: Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+C1007G, by using transient expression systems of HeLa and HEK293 cells. *Pharmacogenet. Genomics*, **15**: 513-522 (2005).
7) Nozawa, T., Minami, H., Sugiura, S., Tsuji, A. and Tamai, I.: Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: *in vitro* evidence and effect of single nucleotide polymorphisms. *Drug Metab. Dispos.*, **33**: 434-439 (2005).
8) Maeda, K., Ieiri, I., Yasuda, K., Fujino, A., Fujiwara, H., Otsubo, K., Hirano, M., Watanabe, T., Kitamura, Y., Kusuhara, H. and Sugiyama, Y.: Effects of organic anion transporting polypeptide 1B1 haplotype on pharmacokinetics of pravastatin, valsartan, and temocapril. *Clin. Pharmacol. Ther.*, **79**: 427-439 (2006).
9) Niemi, M., Schaeffeler, E., Lang, T., Fromm, M. F., Neuvonen, M., Kyrklund, C., Backman, J. T., Kerb, R., Schwab, M., Neuvonen, P. J., Eichelbaum, M. and Kivisto, K. T.: High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1). *Pharmacogenetics*, **14**: 429-440 (2004).
10) Xiang, X., Jada, S. R., Li, H. H., Fan, L., Tham, L. S., Wong, C. I., Lee, S. C., Lim, R., Zhou, Q. Y., Goh, B. C., Tan, E. H. and Chowbay, B.: Pharmacogenetics of SLCO1B1 gene and the impact of *1b and *15 haplotypes on irinotecan disposition in Asian cancer patients. *Pharmacogenet. Genomics*, **16**: 683-691 (2006).
11) Nishizato, Y., Ieiri, I., Suzuki, H., Kimura, M., Kawabata, K., Hirota, T., Takane, H., Irie, S., Kusuhara, H., Urasaki, Y., Urae, A., Higuchi, S., Otsubo, K. and Sugiyama, Y.: Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: Consequences for pravastatin pharmacokinetics. *Clin. Pharmacol. Ther.*, **73**: 554-565 (2003).
12) Niemi, M., Neuvonen, P. J., Hofmann, U., Backman, J. T., Schwab, M., Lutjohann, D., von Bergmann, K., Eichelbaum, M. and Kivisto, K. T.: Acute effects of pravastatin on cholesterol synthesis are associated with SLCO1B1 (encoding OATP1B1) haplotype *17. *Pharmacogenet. Genomics*, **15**: 303-309 (2005).
13) Kitamura, Y., Moriguchi, M., Kaneko, H., Morisaki, H., Morisaki, T., Toyama, K. and Kamatani, N.: Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. *Ann. Hum. Genet.*, **66**: 183-193 (2002).
14) Kozak, M.: Possible role of flanking nucleotides in recognition of the AUG initiator codon by eukaryotic ribosomes. *Nucleic Acids Res.*, **9**: 5233-5252 (1981).
15) Pasanen, M. K., Backman, J. T., Neuvonen, P. J. and Niemi, M.: Frequencies of single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide 1B1 SLCO1B1 gene in a Finnish population. *Eur. J. Clin. Pharmacol.*, **62**: 409-415 (2006).
16) Jada, S. R., Xiaochen, S., Yan, L. Y., Xiaoqiang, X., Lal, S., Zhou, S. F., Ooi, L. L. and Chowbay, B.: Pharmacogenetics of SLCO1B1: Haplotypes, htSNPs and hepatic expression in three distinct Asian populations. *Eur. J. Clin. Pharmacol.*, **63**: 555-563 (2007).
17) Morimoto, K., Oishi, T., Ueda, S., Ueda, M., Hosokawa, M. and Chiba, K.: A novel variant allele of OATP-C (SLCO1B1) found in a Japanese patient with pravastatin-induced myopathy. *Drug Metab. Pharmacokinet.*, **19**: 453-455 (2004).
18) Jung, D., Hagenbuch, B., Gresh, L., Pontoglio, M., Meier, P. J. and Kullak-Ublick, G. A.: Characterization of the human OATP-C (SLC21A6) gene promoter and regulation of liver-specific OATP genes by hepatocyte nuclear factor 1α. *J. Biol. Chem.*, **276**: 37206-37214 (2001).

Drug Metab. Pharmacokinet. 22 (6): *SNP56* (462)-*SNP61* (467) (2007).

SNP Communication

Genetic Variations of VDR/NR1I1 Encoding Vitamin D Receptor in a Japanese Population

Maho UKAJI[1], Yoshiro SAITO[1,2,*], Hiromi FUKUSHIMA-UESAKA[1], Keiko MAEKAWA[1,2], Noriko KATORI[1,3], Nahoko KANIWA[1,4], Teruhiko YOSHIDA[5], Hiroshi NOKIHARA[6], Ikuo SEKINE[6], Hideo KUNITOH[6], Yuichiro OHE[6], Noboru YAMAMOTO[6], Tomohide TAMURA[6], Nagahiro SAIJO[7] and Jun-ichi SAWADA[1,2]

[1]*Project Team for Pharmacogenetics,* [2]*Division of Biochemistry and Immunochemistry,* [3]*Division of Drugs,* [4]*Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan* [5]*Genomics Division, National Cancer Center Research Institute,* [6]*Thoracic Oncology Division, National Cancer Center Hospital, National Cancer Center, Tokyo, Japan* [7]*Deputy Director, National Cancer Center Hospital East, National Cancer Center, Chiba, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: The vitamin D receptor (VDR) is a transcriptional factor responsive to 1α,25-dihydroxyvitamin D_3 and lithocholic acid, and induces expression of drug metabolizing enzymes CYP3A4, CYP2B6 and CYP2C9. In this study, the promoter regions, 14 exons (including 6 exon 1's) and their flanking introns of *VDR* were comprehensively screened for genetic variations in 107 Japanese subjects. Sixty-one genetic variations including 25 novel ones were found: 9 in the 5′-flanking region, 2 in the 5′-untranslated region (UTR), 7 in the coding exons (5 synonymous and 2 nonsynonymous variations), 12 in the 3′-UTR, 19 in the introns between the exon 1's, and 12 in introns 2 to 8. Of these, one novel nonsynonymous variation, 154A>G (Met52Val), was detected with an allele frequency of 0.005. The single nucleotide polymorphisms (SNPs) that increase VDR expression or activity, -29649G>A, 2T>C and 1592(*308)C>A tagging linked variations in the 3′-UTR, were detected at 0.430, 0.636, and 0.318 allele frequencies, respectively. Another SNP, -26930A>G, with reduced *VDR* transcription was found at a 0.028 frequency. These findings would be useful for association studies on *VDR* variations in Japanese.

Key words: ***VDR*****; SNPs; nonsynonymous variation; Japanese**

Introduction

The vitamin D receptor (VDR) is a nuclear receptor, which acts as a transcriptional factor upon binding of the active form of vitamin D, 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], and lithocholic acid.[1-3] Ligand-activated VDR forms a heterodimer with retinoid X receptor, binds to the vitamin D responsive-element and induces expression of its target genes, resulting in partial arrest in G_0/G_1 of the cell cycle, induction of differentiation, or control of calcium homeostatis and maintenance of bone. In addition, VDR has been shown to be involved in induction of drug metabolizing enzymes CYP3A4, CYP2B6 and CYP2C9 in human primary hepatocytes,[2,4] and CYP3A4 in intestinal cell lines.[2,5] VDR and pregnane X receptor share 63% amino acid sequence identity in their DNA binding domains.[2] Like pregnane X receptor and constitutive androstane receptor, VDR transactivates *CYP3A4* through binding to its distal DR3 and proximal ER6 elements.[4,5] Recently, CYP3A4 has been shown to catalyze hydroxylation of

As of August 16, 2007, the novel variations reported here are not found in the database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB Database (http://www.pharmgkb.org/).

This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences from National Institute of Biomedical Innovation and the Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare.

Received; August 17, 2007, Accepted September 21, 2007

**To whom correspondence should be addressed:* Yoshiro SAITO, Ph.D., *Division of Biochemistry and Immunochemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel. +81-3-5717-3831, Fax. +81-3-5717-3832, E-mail: yoshiro@nihs.go.jp*