In the 5′ flanking region, all four detected SNPs (-609C>T, -477T>G, -266C>A, -243G>A) were newly found at relatively high allele frequencies (0.006–0.05). However, these SNPs were not located near the proposed *cis*-regulatory promoter elements (Shestopal et al. 2000). The remaining 21 novel variations were found in intronic regions. Of these SNPs, IVS5–115G>A, IVS12–11G>A, and IVS14-123C>A were detected with allele frequencies of 0.021, 0.038, and 0.155, respectively, but others were rare (<0.01). They were not located in the exon-intron splicing junctions or branch sites.

Seventeen variations were already reported. The ID numbers in the dbSNP databases or references for these SNPs are described in Table 2. The well-known nonsynonymous SNPs, 1627A>G (**5*, Ile543Val), 2194G>A (**6*, Val732Ile), 85T>C (**9*, Cys29Arg), and 1003G>T (**11*, Val335Leu), were found in this study at allele frequencies of 0.283, 0.015, 0.029, and 0.0015, respectively. The allele frequencies of two reported SNPs, 496A>G (Met166Val) and 2303C>A (Thr768Lys), were 0.022 and 0.028, respectively. Recently, 1774C>T (Arg592Trp) was reported from a Korean population (Cho et al. 2007), and its allele frequency was 0.0015 in this study. Nine intronic variations, IVS10-15T>C, IVS13 + 39C>T, IVS13 + 40 G>A, IVS15 + 75A>G, IVS16-94G>T, IVS18-39G>A, IVS21 + 136G>C, IVS22-58G>C, and IVS22-69G>A, and one synonymous variation, 1896T>C (Phe632Phe), were found with various allele frequencies (0.003–0.378, Table 2). The variations previously detected in Japanese (Kouwaki et al. 1998; Yamaguchi et al. 2001; Ogura et al. 2005), 62G>A (Arg21Gln, **12*), 74G>A (His25Arg), 812delT (Leu271X), 1097G>C (Gly366Ala), 1156G>T (Glu386X, **12*), and 1714C>G (Leu572Val), were not found in our study. This might be due to their low frequencies.

Linkage disequilibrium (LD) analysis and haplotype block partition

LD analysis was performed by r^2 and |D′| using 18 SNPs (allele frequency ≥0.01) (Fig. 2). Strong linkages were observed in four pairs of SNPs: between -477T>G and 85T>C (Cys29Arg) ($r^2 = 0.7025$), between 496A>G (Met166Val) and IVS10-15T>C ($r^2 = 0.7964$), between 1627A>G (Ile543Val) and IVS13 + 39C>T ($r^2 = 1.0$), and between IVS14-123C>A and IVS15 + 75A>G ($r^2 = 1.0$). In addition, two known rare SNPs, IVS22-69G>A (rs290855) and IVS22-58G>C (rs17116357), were perfectly linked ($r^2 = 1.0$) (data not shown). As for |D′| values, only 43 pairs (28%) out of 153 pairs gave |D′| = 1.0, indicating that a number of recombinations had occurred within this gene. This is not surprising because *DPYD* is a huge gene of at least 950 kb in length with 3 kb of coding sequences. However, it was difficult to estimate past recombination events in *DPYD* from our data alone because our variations were mostly limited to exons and surrounding introns.

To define haplotype blocks, we utilized the HapMap data because SNPs were comprehensively genotyped with an average density of 1 SNP per 1.8 kb. Of 1,002 variations of *DPYD* genotyped by the HapMap project, 474 SNPs were polymorphic for 44 unrelated Japanese subjects. When the LD profiles for Japanese were obtained by Marker using the HapMap data, strong LD (|D′| > 0.75) clearly decays within introns 11, 12, 13, 14, 16, 18, and 20 (data not shown), suggesting that recombination had occurred in these regions. Based on these findings, the SNPs detected in our study were divided into six haplotype blocks (Figs. 1, 2). Block 1, the largest block, ranges from the 5′-untranslated region (5′-UTR) to intron 10 (347 kb), and includes 22 variations. Block 2 includes eight variations from IVS12-11G>A in intron 12 to IVS13 + 40G>A in intron 13. Block 3 includes six variations from IVS13-47_48insTA in intron 13 to IVS14 + 100T>G in intron 14. Block 4 contains only three SNPs, IVS14-123C>A, IVS14-21C>A and IVS15 + 75A>G, and ranges from intron 14 to intron 15. Block 5 consists of IVS16-94G>T and four rare variations from intron 16 to exon 18. Although the HapMap data showed a decline in LD in intron 20, we defined a block ranging from intron 18 to intron 22 as block 6 because only rare variations (allele frequencies <0.01) were detected downstream of intron 20 (exon 21, intron 21, and intron 22). The block partitioning based on the HapMap data fitted our SNPs well: more than 70% of SNP pairs in each block (block 1–6) gave pair-wise |D′| values greater than 0.8 (Fig. 2).

Haplotype estimation

Using 22, 8, 6, 3, 5, and 11 variations in blocks 1 to 6, 23 (block 1), 8 (block 2), 7 (block 3), 3 (block 4), 6 (block 5), and 11 (block 6) haplotypes were identified or inferred (Fig. 3). Probabilities of diplotype configurations in all six blocks were 100% for over 97% of the subjects. To discriminate our block haplotypes from the previously assigned alleles or haplotypes (*DPYD*1* to **13*), the mark, #, was used to indicate block haplotypes.

In block 1, the most dominant haplotype without any variation was *#1a* (0.818 in frequency), followed by *#1b* (0.045), *#9c* (0.022), and *#1c* (0.021). As suggested by LD (Fig. 2), *#9c*, the major subtype of the *#9* group bearing 85T>C (Cys29Arg), also harbored -477T>G in the 5′-UTR. Known nonsynonymous SNP, 496A>G (Met166Val), was assigned to three haplotypes, *#9d*, *#166Va*, and *#166Vb*.

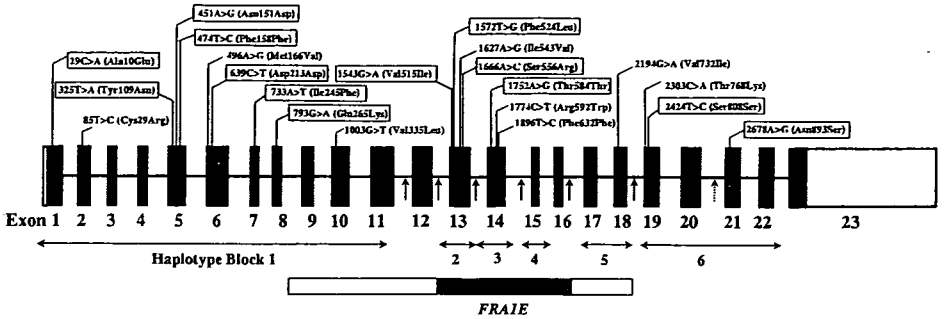


Fig. 1 Twenty-one variations detected in the coding exons are depicted in the schematic diagram of the *DPYD* gene. Fourteen novel variations are enclosed by *squares*. The recombination spots were estimated based on the LD profiles obtained from Japanese data in the HapMap project and indicated by *arrows*. The borders (between introns 8 and 18 of the *DPYD*) and core region (between introns 12 and 16) of *FRA1E* identified by Hormozian et al. (2007) are indicated as an *open* and *closed box*, respectively

In block 2, four haplotypes, #*1a* (0.529), #*5a* (0.245), #*1b* (0.176), and #*5b* (0.038), were major in Japanese and accounted for 99% of all inferred haplotypes. Two subtypes of the #*5* group, #*5a* and #*5b*, both of which harbored Ile543Val (**5*) and IVS13 + 39C>T, were distinguished by a novel intronic SNP, IVS12-11G>A.

As for block 3, in addition to #*1a* (0.848), #*1b* harboring the synonymous SNP, 1896T>C (Phe632Phe), was found at a relatively high frequency (0.138).

Block 4 is simple and comprises only three haplotypes, #*1a* (0.845), #*1b* (0.154) and #*1c* (0.0015). The second frequent haplotype, #*1b*, harbored perfectly linked SNPs, IVS14-123C>A and IVS15 + 75A>G.

Block 5 contained IVS16-94G>T, the most frequent SNP among the 55 SNPs found in this study, which was assigned to #*1b* with a frequency of 0.374. This block also contained the known nonsynonymous SNP, 2194G>A (Val732Ile, **6*), which was assigned to #*6a* (0.015).

In block 6, the most dominant haplotype was #*1a* (0.915). It was followed by #*1b* (0.032) with IVS18-39G>A and #*768K* (0.028) with 2303C>A (Thr768Lys).

The HapMap data include nine SNPs that we detected (Table 2). Of them, six, 85T>C (rs1801265), 496A>G (rs2297595), 1627A>G (rs1801159), 1896T>C (rs17376848), IVS16-94G>T (rs7556439) and IVS18-39G>A (rs12137711), were suitable for haplotype tagging SNPs (htSNPs) to capture the block haplotypes, block 1 #*9*, block 1 #*166V*, block 2 #*5*, block 3 #*1b*, block 5 #*1b*, and block 6 #*1b*, respectively. IVS21 + 136G>C (rs11165777) and IVS22-69G>A (rs290855)/IVS22-58G>C (rs1711 6357), were the marker SNPs for block 6 #*1e* and #*1f*, respectively, but very rare (allele frequencies = 0.003) in Japanese. The six SNPs, especially 85T>C (rs1801265) and 496A>G (rs2297595), were in strong LD ($r^2 > 0.8$) with other HapMap SNPs in Japanese (Table 3), indicating that many HapMap SNPs were concurrently linked on the same haplotypes.

Next, the combinations of block haplotypes (inter-block haplotypes) were analyzed focusing on the haplotypes with frequencies of >0.01 in each block (Fig. 4). Between blocks 1 and 2, both #*1a* and #*1b* in block 1 were complicatedly associated with various haplotypes in block 2. It should be noted that #*9c* in block 1 was linked either with block 2 #*1b* (0.016 in absolute frequency) or with block 2 #*5a* (0.006, not shown in Fig. 4). #*1c* in block 1 was completely linked with block 2 #*1a*. #*151D* in block 1 (not shown in Fig. 4), which was a rare haplotype (0.009) harboring 451A>G (Asn151Asp), was completely linked with #*5a* in block 2.

Between blocks 2 and 3, both #*5b* and #*1b* in block 2 were mostly linked with #*1a* in block 3, whereas both #*1a* and #*5a* in block 2 were complicatedly linked with #*1a*, #*1b*, or other rare haplotypes such as #*1c* (not shown in Fig. 4) in block 3. Between blocks 3 and 4 and between blocks 4 and 5, no strong associations of block haplotypes were observed except for the linkage of block 5 #*6a* to block 4 #*1a*. Between blocks 5 and 6, most of #*1b* and all of #*6a* in block 5 were linked with #*1a* in block 6. Although #*1a* in block 6 was associated with various haplotypes in block 5, #*1b* in block 6 was completely linked with #*1a* in block 5.

Among the six blocks, the following combinations were major: #*1a* (block 1)–#*1a* (block 2) –#*1a* (block 3)–#*1a* (block 4)–#*1a* (block 5)–#*1a* (block 6) (0.239 in frequency), #*1a*–#*5a*–#*1a*–#*1a*–#*1b*–#*1a* (0.081), #*1a*–#*1a*–#*1a*–#*1a* –#*1b*–#*1a* (0.075), #*1a*–#*5a*–#*1a*–#*1a*–#*1a*–#*1a* (0.070), #*1a*–#*1b*–#*1a*–#*1a*–#*1a*–#*1a* (0.060) and #*1a*–#*1a*–#*1b*–#*1a*–#*1a*–#*1a* (0.051).

Ethnic differences in distributions of *DPYD* SNPs and haplotypes

We compared SNP and haplotype distributions in Japanese with those in other ethnic groups reported in the literature

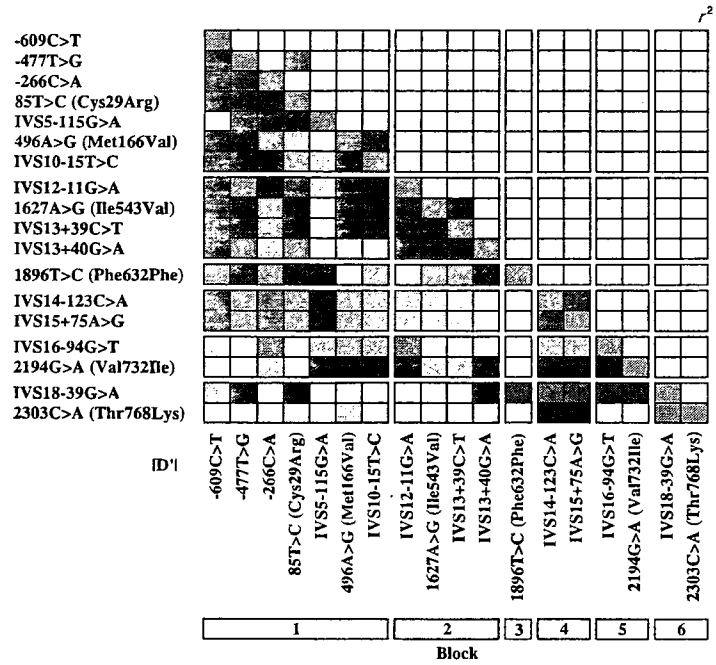


Fig. 2 Linkage disequilibrium (*LD*) analysis of *DPYD*. Pairwise LD between 18 common SNPs (>0.01 in allele frequencies) is expressed as r^2 (*upper*) and |D'| (*lower*) by a 10-graded *blue color*. The *denser color* indicates higher linkage. The haplotype block partition based on LD measure |D'| of HapMap data in Japanese is also indicated

or HapMap project. Notably, IVS14 + 1G>A (*2), 1897delC (Pro633GlnfsX5, *3), 1601G>A (Ser534Asn, *4), 295_298delTCAT (Phe100SerfsX15, *7), 703C>T (Arg235Trp, *8), 2983G>T (Val995Phe, *10), 62G>A (Arg21Gln, *12), 1156G>T (Glu386X, *12), and 1679T>G (Ile560Ser, *13) were not found in this study. Furthermore, several SNPs showed marked differences in allele frequencies among Japanese and other ethnic groups (Table 4).

The allele frequency of 85T>C (Cys29Arg, *9), the tagging SNP for block 1 #9, was quite different between Asians and Caucasians. Its allele frequency in Japanese (0.029 in this study) and Taiwanese (0.022) (Hsiao et al. 2004) was much lower than that in Caucasians (0.185–0.194) (Seck et al. 2005; Morel et al. 2006).

The SNP 496A>G (Met166Val) in block 1 is found at a lower allele frequency in Japanese (0.022) than in Caucasians (0.080) (Seck et al. 2005). Seck et al. (2005) inferred two haplotypes harboring 496A>G (Met166Val) from 157 Caucasians: *hap5* (#9d in this study) harboring additional 85T>C (Cys29Arg) and IVS10-15T>C and *hap11* concurrently harboring IVS10-15T>C alone with frequencies of 0.040 and 0.014, respectively. In our haplotype analysis, #166Va (0.012) corresponding to *hap11* (0.014) was found with a similar frequency in Japanese, whereas the frequency of #9d (0.006) was much lower than that of the corresponding haplotype, *hap5* (0.040) in Caucasians.

1627A>G (Ile543Val, *5) in block 2 was found with comparable allele frequencies among Japanese (0.283 in this study), Caucasians (0.14-0.275) (Seck et al. 2005; Ridge et al. 1998a), African-Americans (0.227) (Wei et al. 1998), and Taiwanese (0.210–0.283) (Wei et al. 1998; Hsiao et al. 2004).

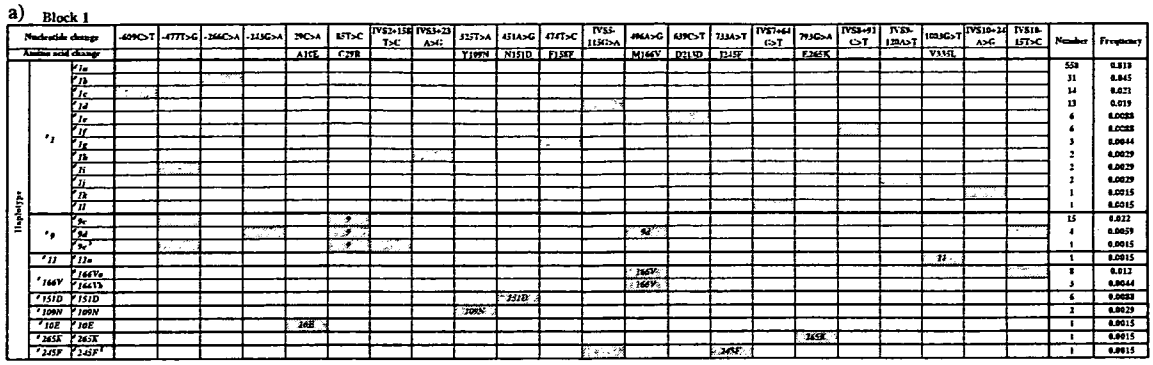
The allele frequency (0.015) of 2194G>A (Val732Ile, *6) in block 5 in our Japanese population is slightly lower than that previously reported in Caucasians (0.022-0.058) (Seck et al. 2005; Ridge et al. 1998a) and Finish (0.067) (Wei et al. 1998), but is comparable to that in Taiwanese (0.012-0.014) (Wei et al. 1998; Hsiao et al. 2004) and African-Americans (0.019) (Wei et al. 1998).

Ethnic differences in the allele frequencies were also observed with synonymous and intronic variations (Table 4). The allele frequency of 1896T>C (Phe632Phe), which tags block 3 #1b, was higher in Japanese (0.139 in this study) than in Caucasians (0.035) (Seck et al. 2005). *Hap13* assigned in 157 Caucasians by Seck et al. (2005) is the counterpart of block 3 #1b, and its frequency (0.012) was much lower than that in Japanese (0.138).

In contrast, IVS10-15T>C linked to 85T>C (*9) or 496A>G (#166V) within block 1 showed a lower allele frequency in Japanese (0.018) than in Caucasians (0.127). Seck et al. (2005) assigned *hap7* as the haplotype containing IVS10-15T>C alone with a haplotype frequency of 0.03 in Caucasians. In Japanese, however, the corresponding haplotype was not found.

Allele frequencies of IVS18-39G>A and IVS22-69G>A, which are tagging SNPs for block 6 #1b and #1f, respectively, are lower in Japanese (0.032 and 0.003, respectively) than in Caucasians (0.105 and 0.183, respectively).

a) Block 1



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					5				167	0.245
		*5b					5				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+34del T	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					6	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												19	0.028
	*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

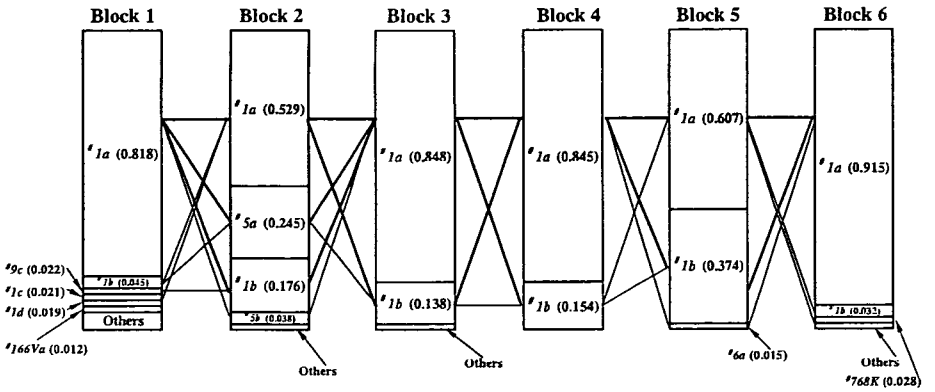


Fig. 4 The combinations of block haplotypes in Japanese. *Thick lines* represent combinations with frequencies over 10%, and *thin lines* represent combinations with frequencies of 1.0–9.9%

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,

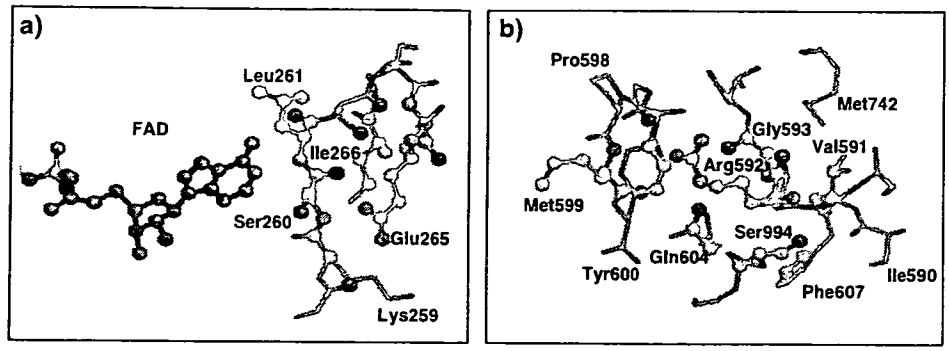


Fig. 5 Stereo view of the variation sites in pig DPD (accession code of the Protein Data Bank: 1gth). Glu265 (a), Arg592 (b) and their adjacent residues are shown as *ball-and-stick* models with oxygens in *red*, nitrogens in *blue*, carbons in *gray* and sulfur in *yellow*. The adenosine moiety of the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

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消化器毒性

Toxicity of digestive system associated with chemotherapy

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●要旨●抗癌剤による消化器毒性は主に嘔気・嘔吐と粘膜炎（口内炎，下痢）があげられ，これらのマネージメントは治療を維持するうえでたいへん重要である。米国臨床腫瘍学会（ASCO）やNational Comprehensive Cancer Network（NCCN）のガイドラインでは抗癌剤の催嘔吐性を4段階に分類し，それぞれ予防的にステロイドやセロトニン受容体拮抗薬を使用する方法を示しており，それに準じて十分に制吐剤を使用する。また粘膜毒性は時に重篤となるため骨髄抑制の併存などに注意し，必要であれば入院とし止痢薬の投与や腸管安静・点滴を十分に行う必要がある。

● key words：嘔気・嘔吐，口内炎，下痢

はじめに

嘔気，嘔吐などの消化器毒性は患者にとって抗癌剤の副作用の代名詞のようなものであり，また下痢などの副作用に骨髄抑制を併発すると，重篤な有害事象となり治療関連死亡を引き起こすこともある。消化器毒性のマネージメントは抗癌剤治療継続にとってたいへん重要である。NCCNやASCOのガイドラインを中心に，消化器毒性の実際的な対処法について解説する。

嘔気・嘔吐

1. 嘔気・嘔吐の発生起序，分類

抗癌剤による嘔気・嘔吐は，①化学受容器引金帯（chemoreceptor trigger zone；CTZ），②咽頭や消化管の迷走神経，③大脳皮質からの3つの経路で延髄にある嘔吐中枢が刺激されると引き起こされる。嘔気を誘発する神経伝達物質としてセロトニン（5-hydroxytriptamine；5-HT_3），ドパミン，ニューロキニン（neurokinin-1；NK-1）などがあげられる。

抗癌剤によって引き起こされる嘔気・嘔吐は急性，遅発性，予測性の3つに分けられる。急性悪心・嘔吐は通常薬剤開始から24時間以内に起こるものを指し，遅発性嘔吐は24時間以後に起こるものを指す。多くは48～72時間ごろに高度となり，7日までに消失する。予測性嘔吐は抗癌剤投与による嘔気・嘔吐の経験があり，次回投与前に起こるものを指す。

抗癌剤の催嘔吐性は急性嘔吐が出現する割合によりhigh（>90%），moderate（30～90%），low（10～30%），minimal（<10%）の4つのリスクに分けられる。2006年に改訂された米国臨床腫瘍学会（ASCO）のガイドラインにあげられている薬剤を表1に示す[1]。

2. 嘔気・嘔吐に対する薬物療法の実際

使用する抗癌剤の催嘔吐性に合わせて，制吐剤を組み合わせる。併用療法の場合にはもっともリスクの高い薬剤に対する治療を選択する。制吐剤は5-HT_3受容体拮抗薬・ステロイドを中心に使用し，患者の状態に合わせてその他薬剤を組み合わせる[2]。米国では2003年にNK-1受容体拮抗薬（aprepitant）が承認されhigh，moderate risk群に使用されているが，本邦では現在治験中である。

high risk，moderate riskの抗癌剤を使用する場合には，5-HT_3受容体拮抗薬，デキサメタゾンを組み合わせる。high risk群では初日の抗癌剤投与前に

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表1　抗癌剤催嘔吐性の分類

High	Moderate	Low	Minimal
cisplatin	carboplatin	5-FU	bevacizumab
cyclophosphamide ≧1500mg/sqm	cyclophosphamide＜1500mg/sqm	capecitabine	bleomycin
dacarbazine	doxorubicin	cetuximab	gefitinib
streptozotocin	epirubicin	etoposide	sunitinib
	imatinib	docetaxel	
	irinotecan	gemcitabine	
	oxaliplatin	methotrexate	
		mitomycin	
		paclitaxel	
		pemetrexed	
		topotecan	

〔文献1）より引用〕

表2　制吐剤使用の実際（催嘔吐性分類別）

化学療法開始前		Day 2，3	Day 4
High			
デキサメタゾン	20mg 内服または点滴静注	16mg 内服（または点滴静注）	16mg 内服
および			
5-HT_3受容体拮抗薬	内服または点滴静注		
（ロラゼパム内服）			
Moderate			
デキサメタゾン	8 mg 点滴静注	8 mg 内服（または点滴静注）	なし
および		または	
5-HT_3受容体拮抗薬	内服または点滴静注	内服	
Low			
デキサメタゾン	8 mg　内服または点滴静注	なし	なし
Minimal			
予防的投与不要			

〔文献2）より引用〕

5-HT_3受容体拮抗薬を投与し，デキサメタゾン（デカドロン®）を静注または経口で1日目20mg（aprepitant 使用時は12mg），2～4日目までは16mg（aprepitant 使用時は8 mg）を使用する（表2）。moderate risk 群では初日は5-HT_3受容体拮抗薬およびデキサメタゾン8 mg にて予防投与を行い，2～3日には5-HT_3受容体拮抗剤またはデキサメタゾン8 mg を使用する。low　risk 群では1日目にデキサメタゾン8 mg を使用する。minimal risk 群では予防的な制吐剤の使用は不要である。5-HT_3受容体拮抗薬の使用量を表3に示した。

予防的制吐剤の使用によっても嘔気，嘔吐が出現した場合にはドパミン拮抗薬であるメトクロプラミド（プリンペラン®），フェノチアジン系のプロクロルペラジン（ノバミン®），ブチロフェノン系のハロペリドール（セレネース®），ベンゾジアゼピン系のロラゼパム（ワイパックス®）など異なる種類の制吐剤を追加する。また遅発性嘔吐に関しても予防が重要であり，ステロイドと5-HT_3受容体拮抗薬の投与でも嘔気が出現する場合には，定期的に制吐剤を追加使用することが有効である。

予測性嘔吐の予防にもっとも重要なことは，治療ごとに有効な制吐剤を用いて嘔気を予防することである。また音楽やアロマなどのリラクゼーションの導入も検討する。予測性嘔吐に対する薬物療法としては，ベンゾジアゼピン系抗不安薬（アルプラゾラム；ソラナックス®，ロラゼパム；ワイパックス®）を前日夜や当日朝に内服させると有効である。

表３　5-HT$_3$受容体拮抗薬の使用量

5-HT$_3$ serotonin receptor antagonists		注（１日１回）	内服（１回量）
azasetron	セロトーン®	10mg （１日２回まで可）	10mg　１日１回
granisetron	カイトリル®	3mg （１日２回まで可）	2mg　１日１回 （増減可）
indisetron	シンセロン®		8mg　１日１回
odansetron	ゾフラン®	4mg （追加投与可）	4mg　１日１回 （増減可）
ramosetron	ナゼア®	0.3mg （１日２回まで可）	0.1mg（OD錠）　１日１回
tropisetron	ナボバン®		5mg（カプセル）　１日１回

口内炎

口内炎は抗癌剤の直接作用により炎症性サイトカインや活性酸素が放出され，口腔粘膜細胞が傷害され出現する。また抗癌剤による好中球減少による口腔内感染が二次的に関与している。抗癌剤投与前からの口腔歯肉炎や口腔内感染症が発症に関与しており，口腔内感染症の治療や口腔内衛生管理が発症予防に重要である[3]。口内炎を起こしやすい抗癌剤としては，5-フルオロウラシル（5-FU），メトトレキサート，エトポシド，シタラビン，ドキソルビシンなどがあげられる。

1.　口内炎予防に対する薬物療法

口内炎予防に対する方法として口腔内冷却法（cryotherapy）やアロプリノール含嗽があげられる。口腔内冷却法は氷片により口腔内血管を収縮させ，抗癌剤の口腔粘膜への移行を減少させ口内炎を予防する方法である。5-FUの急速静注の５～10分前から30分後まで氷片で冷やすことでGrade 3～4の口内炎を軽減させることが報告されている。またアロプリノール含嗽は5-FUによる口内炎を軽減させる効果がいくつかの少数例で報告されているが，比較試験では有効性が示されなかった。その他クロルヘキシジン，スクラルファート，G-CSF・GM-CSFなどの口内炎軽減効果が報告されているが少数例の報告であり，結果がさまざまであるため標準的な治療とはなっていない。

実際的には歯周病やカンジダなどの真菌感染症を化学療法前に治療し，化学療法中もうがいなどで口腔内を清潔に保つよう指導する。

2.　口内炎に対する薬物療法

軽度の口内炎の場合は抗炎症薬であるアズレン（ハチアズレ®，アズノール®）や口腔用ステロイド軟膏（デキサルチン®，ケナログ®），口腔内ステロイド貼付錠（アフタッチ®）などを使用し，刺激の強い食事を避けるよう指導する。疼痛が強い場合にはキシロカイン®（ビスカス）での含嗽や消炎鎮痛薬の内服を，潰瘍形成が強い場合にはエレース® 含嗽を試みる。

下　痢

1.　下痢の発症起序

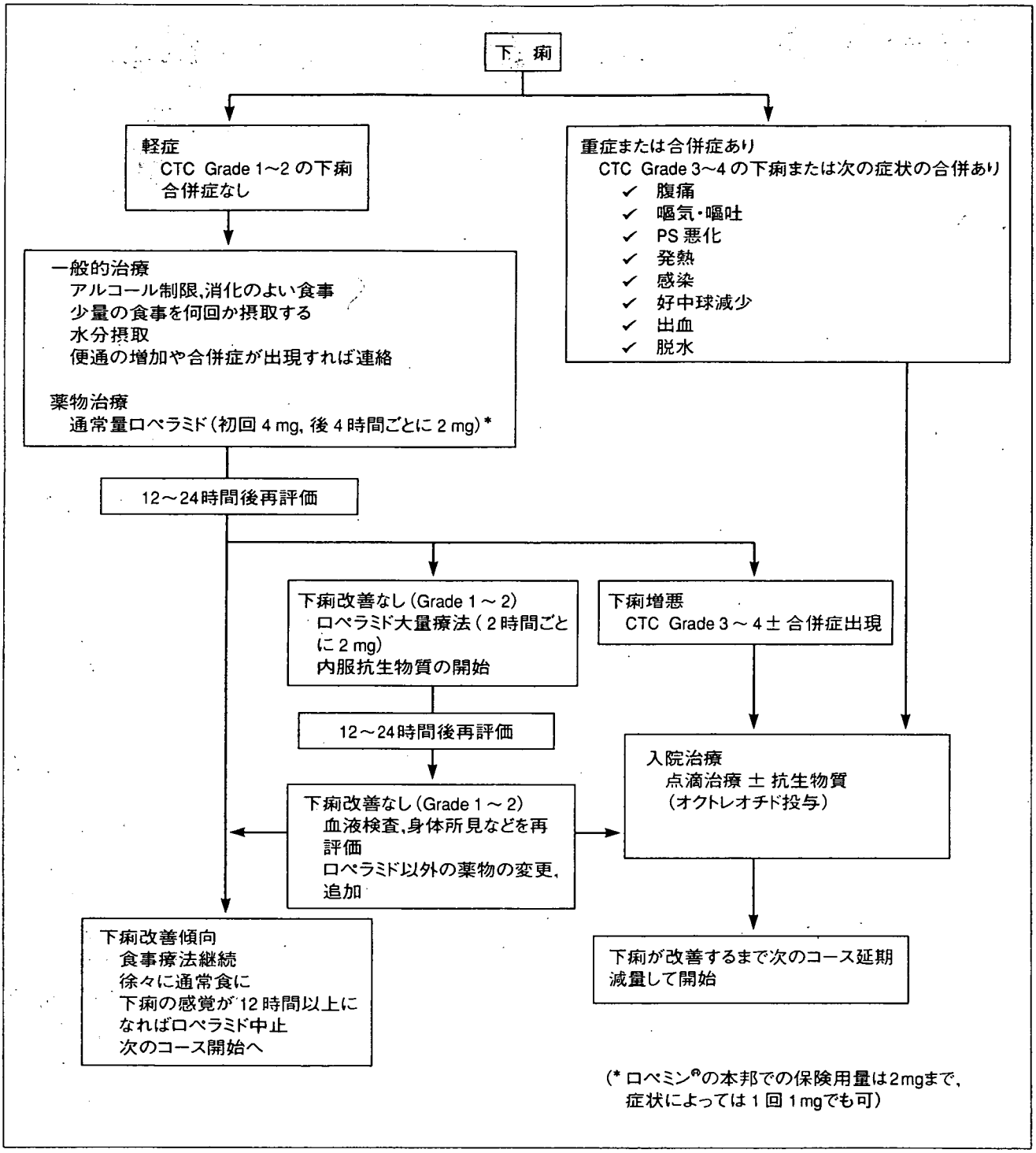
下痢を起こしやすい薬物としては5-FU，イリノテカン，メトトレキサートなどがあげられる。抗癌剤に起因する下痢は早期に起こるコリン作動性に生じる下痢と，投与後数日～２週ぐらいで生じる抗癌剤の腸管粘膜障害による下痢の大きく２種類に分けられる。

イリノテカンはコリン作動性による早発性の下痢を引き起こす代表的な薬剤で，投与後蠕動亢進，発汗などとともに発症する。前投薬に硫酸アトロピンを加えることでコントロール可能である。遅発性下痢はイリノテカンの容量制限毒性で，投与後数日～２週間のうちに発症する。イリノテカンの代謝酵素であるuridine diphosphoglucuronosyl transferase 1A1（UGT1A1）の遺伝子変化による活性の低下があると，イリノテカンの活性体SN-38の代謝が遅延し下痢などの副作用が重症化することが報告されている。

5-FUによる下痢はロイコボリン（LV）との併用により増強される。5-FUの代謝酵素であるdihydropyrimidine dehydrogenase（DPD）欠損のある患者では強い副作用が出現し，骨髄抑制や口内炎，下痢などの合併により治療関連死亡の可能性があり注意が必要である。

2.　下痢に対する治療

抗癌剤による下痢の治療の基本は対症療法である。腸管に負担をかけないような食事に変更し，症状が強い場合は腸管安静（絶食）とし，点滴で水分，電解質補給を十分に行う。嘔吐，発熱，脱水，骨髄抑制などを合併する場合には入院のうえ治療を行う（図1）。



〔文献4〕より引用〕

図 1　抗癌剤起因性下痢の対処

薬物療法としてはロペラミド（ロペミン®），オクトレオチド（サンドスタチン®），アヘンチンキ，ビスマス製剤，タンニン酸アルブミン（タンナルビン®）などがあげられる。

ロペラミドは腸管運動を抑制すると同時に，腸管における水分・電解質の分泌を抑止し吸収を促進させ強力な止痢作用を有している。ロペラミド大量療法はロペラミド 2 mg を下痢症状の発現とともに 2 時間ごとに服用する方法で，イリノテカンによって引き起こされる下痢のほとんどが 3 日以内にコントロール可能であったと報告されている。

オクトレオチドはソマトスタチンの合成アナログ

で，下痢を引き起こすvasoactive intestinal peptide（VIP）などの消化管ホルモンの分泌を抑えたり，腸管分泌の抑制・吸収促進を促したりすると考えられている。ASCOのガイドラインではオクトレオチド100～150μgを1日3回皮下注射または25～50μg/hr持続点滴の使用が勧められているが，本邦では下痢に対する使用は保険適応外である。

その他症状に合わせてコデインやビスマス，タンニン酸アルブミンなどを併用し，発熱や骨髄抑制を合併する場合にはG-CSFや抗生物質の投与を考慮する。

おわりに

消化器癌で多く使用する抗癌剤は比較的強い消化器毒性を有し，治療の継続を左右するため適切な対処が重要である。これらの症状は原病の増悪でも認められることがあり，コントロール可能であった副作用が強くなった場合には，脳転移や腹膜病変の評価も同時に必要である。

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Abdominal Sacral Resection for Posterior Pelvic Recurrence of Rectal Carcinoma: Analyses of Prognostic Factors and Recurrence Patterns

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Background: Local recurrence of rectal cancer presents challenging problems. Although abdominal sacral resection (ASR) provides pain control, survival prolongation, and possibly cure, reported morbidity and mortality are still high, and survival is still low. Thus, appropriate patient selection and adjuvant therapy based on prognostic factors and recurrence patterns are necessary. The purpose of this study was to evaluate the results of ASR for posterior pelvic recurrence of rectal carcinoma and to analyze prognostic factors and recurrence patterns.

Methods: Forty-four patients underwent ASR for curative intent in 40 and palliative intent in 4 cases. All but one could be followed up completely. Multivariate analyses of factors influencing survival and positive surgical margins were conducted.

Results: Morbidity and mortality were 61% and 2%, respectively. Overall 5-year survival was 34%. The Cox regression model revealed a positive resection margin (hazard ratio, 10 [95% confidence interval, 3.8–28]), a local disease-free interval of < 12 months (4.2 [1.8–9.8]), and pain radiating to the buttock or further (4.2 [1.6–11]) to be independently associated with poor survival. The logistic regression model showed that macroscopic multiple expanding or diffuse infiltrating growths were independently associated with a positive margin (7.5 [1.4–40]). Of the patients with recurrence, 56% had failures confined locally or to the lung.

Conclusions: ASR is beneficial to selected patients in terms of survival. To select patients, evaluation of the resection margin, the local disease-free interval, pain extent, and macroscopic growth pattern is important. To improve survival, adjuvant treatment should be aimed at local and lung recurrences.

Key Words: Therapy—Surgery—Rectal cancer—Local recurrence—Recurrence—Prognostic factor.

Posterior pelvic recurrence[1–3] (PPR) of rectal carcinoma, which involves the sacrum and/or sacral nerves, presents challenging clinical problems. It may cause sacral nerve pain, perineal ulcers, fistula formation, bleeding, bowel and/or urinary tract obstruction, sepsis, and, finally, death.[4] These conditions are difficult to treat, and chemotherapy provides only minimal benefits at present.[4–6] Radiotherapy may give pain relief, but its effectiveness is limited and temporary.[4,7–9] Conventional abdominoperineal resection or local excision is only palliative.[10,11]

In 1981, Wanebo and Marcove[11] reported the advantage of the abdominal sacral resection (ASR), which was first described by Brunschwig and Barber[12] in 1969, for PPR of rectal carcinoma. Although published data on this operation are still limited and

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there have been few long-term follow-up studies, this aggressive operation provides pain control, prolongation of survival, and possibly cure.[13–22] However, reported morbidity and mortality are significantly high,[13–22] and survival is still low.[13–22] Therefore, appropriate selection of patients, especially with reference to the probable prognosis, is necessary. In addition, adjuvant therapy based on recurrence patterns may be required. The purpose of this study was to evaluate the results of ASR for PPR of rectal carcinoma and to analyze prognostic factors and recurrence patterns.

PATIENTS AND METHODS

Between March 1983 and May 2000, 44 patients with PPR of rectal carcinoma that involved the sacrum on computed tomography (CT) were considered candidates for ASR and admitted to the National Cancer Center Hospital, Tokyo. There were 35 men and 9 women, with a median age of 55 years (range, 32–73 years). Of these, 40 patients underwent initial operation at other hospitals. Selection criteria for curative-intent ASR were as follows: (1) medical fitness for ASR; (2) no signs of disseminated disease on preoperative imaging; (3) tumors involving the sacrum but not the first sacral bone and the bony lateral walls; and (4) tumors anatomically confined within the pelvis, with or without resectable solitary liver metastasis. The imaging studies routinely performed before resection were abdominal and pelvic CT, abdominal ultrasonography, and chest roentgenogram until 1989; pelvic magnetic resonance imaging and chest CT were added thereafter.

Of the 44 patients for whom ASR was attempted, 40 received curative-intent ASR, and 4 received palliative-intent ASR because of 1 or 2 lung metastases in 3 and 3 liver metastases in 1. Of the 40 who received curative-intent ASR, 33 patients underwent macroscopic curative ASR, 2 with solitary liver metastasis underwent macroscopic curative ASR with complete resection of liver metastasis, 1 with 4 peritoneal metastases adjacent to the main tumor underwent macroscopic curative ASR with complete resection of peritoneal metastases, and the remaining 4 underwent palliative ASR because of macroscopic residual local tumor in 3 and residual lymph node metastases in 1. Of the four who received palliative-intent ASR, three with lung metastases underwent palliative ASR leaving only residual lung metastases in two and both residual lung and local tumors in one, and one with three liver metastases underwent macroscopic curative ASR with complete resection of liver metastases. Conseuently, 37 underwent macroscopic curative resection, and 7 underwent macroscopic palliative resection. Of them, 27 patients received no radiation, 13 received preoperative adjuvant radiation of 30 to 73 Gy (median, 44 Gy), and 4 received 44 to 50 Gy (median, 50 Gy) as previous treatment.

Data for these patients were collected and entered prospectively into the database of the Colorectal Surgery Division. They included the following: (1) patient demographics; (2) treatment and pathology of the primary rectal cancer; (3) presentation of PPR; (4) treatment and pathology of recurrent tumor; (5) operative details; (6) hospital course, including complications; and (7) outcome. Of these, 15 variables were selected for prognostic factor analysis (Table 1) by consideration of their potential relationship to survival after ASR, as indicated by previous studies.[13–15,17–19,22] The local disease–free interval (LDFI) was defined as the interval between the initial curative operation and the occurrence of symptoms or detection of asymptomatic PPR by CT.

Surgical Procedure

Our surgical procedure was basically similar to that originally described by Wanebo and Marcove[11] and Wanebo et al.;[13] however, it was slightly modified.[23] Our sacral resection was performed immediately after the abdominal phase as a one-stage procedure instead of a two-stage procedure.[13] The presence of liver metastasis did not preclude continuation of the procedure if it was solitary and if the disease-free interval was sufficiently long. Solitary liver metastasis was resected simultaneously. We did not make full-thickness fascial myocutaneous flaps for sacroperineal wound closure but sutured the wound simply because there were no patients with large exposed tumors at the perineum.

After the patient was placed in a supine position with flexed and abducted thighs, dissection was started at the aortic bifurcation, and the common and external iliac vessels were dissected. The internal iliac vessels were divided at their root or beyond the superior gluteal artery. Adipose tissue, lymphatics, and the nodes surrounding these vessels, including obturator nodes, were removed completely, and the muscular pelvic side walls and the sacral nerve roots were exposed. The upper limit of the tumor was identified, and the anterior surface of the sacrum was dissected down to the planned level of sacral transection. When the tumor adhered or invaded into

TABLE 1. *Univariate Predictors of Adverse Outcome*

Variable	No. of Patients	Overall survival (%)			*P*
		1-yr	3-yr	5-yr	
Overall	44	90	47	34	
Gender					
Female	9	87	45	45	.41
Male	35	91	48	32	
Age					
< 60 years	30	96	55	40	.10
≥ 60 years	14	92	31	23	
Primary cancer stage					
I, II	2, 13	93	64	48	.046
III	22	90	39	31	(I, II, III vs. IV)
IV	7	85	28	14	
Initial surgery					
Local excision, anterior resection	1, 20	90	51	36	.83
Abdominoperineal resection	23	90	44	34	
Initial lymphadenectomy					
Conventional	33	93	55	41	.25
Extended	11	81	27	18	
Local-disease-free interval (months)					
≤ 12	17	75	20	20	.0042
> 12	27	96	62	43	
Preoperative CEA level (ng/ml)					
≤ 10	23	91	70	49	.025
> 10	21	90	25	20	
Extent of preoperative pain					
None, perineum	15, 17	93	55	43	.0006
Buttock	7	85	35	0	(none, perineum vs. buttock, more)
Thigh, leg	3, 2	50	0	0	
Tumor extent					
Solitary pelvic tumor	24	95	55	40	.17
Pelvic metastasis	12	75	43	29	(solitary tumor vs. others)
Distant metastasis	8	85	28	28	
Largest tumor diameter (cm)					
≤ 5	26	92	50	40	.086
> 5	18	88	40	24	
Sacral involvement					
Adhesion	27	84	56	37	.85
Periosteum, marrow	11, 6	94	32	32	
Resection margin					
Microscopic negative	24	95	81	62	< .0001
Microscopic positive	13	91	16	8	(microscopic negative vs. others)
Gross positive, residual	7	71	0	0	
Pathological grade					
Well, moderate	4, 29	90	40	35	.49
Mucinous, adenosquamous	6, 1	85	57	42	(poor, signet vs. others)
Poor, signet-ring cell	3, 1	75	75	0	
Macroscopic growth pattern					
Solitary expanding	15	92	70	70	.0027
Multiple expanding	5	80	40	20	(solitary vs. others)
Diffuse infiltrating	24	87	34	13	
Preoperative radiation					
Yes	13	91	55	46	.55
No	31	90	44	29	

CEA, carcinoembryonic antigen.

urogenital organs, the remaining rectum, pelvic nerves or muscles, and involved organs were all resected en bloc to avoid incomplete resection and cancer cell spillage. To facilitate resection and hemostasis and to shorten operating time, a combined abdominal and perineal approach was used.

After dissection of the lateral, cephalad, anterior, and caudal aspects of the tumor with surrounding organs to be resected was accomplished, the patient was placed in a prone position with flexed and abducted thighs. A posterior sacral incision including the perineal lesion was made, and the sacrum and

gluteal muscles were exposed. The gluteal muscles, sacrotuberous ligament, sacrospinous ligaments, and pyriformis muscles were divided as far from the tumor as possible. After the level of abdominal dissection and the extent of the tumor were confirmed by hand in the pelvic cavity, a laminectomy proximal to the planned level of sacral transection was performed to preserve the noninvolved sacral nerve roots and ligate the dura. The sacrum was transected by an osteotome, and en-bloc resection of the tumor with the sacrum and the surrounding organs was accomplished. The gluteal muscles and skin were closed primarily. Again, the patient was placed in a supine position with flexed and abducted thighs. A colostomy and an ileal conduit were made.

Extent of Resection

Levels of sacral transection included S2 in 6 patients, S2–3 in 19, S3 in 5, S3–4 in 11, S4 in 1, and S4–5 in 2. Thirty-nine patients underwent total pelvic exenteration, one underwent posterior pelvic exenteration, and four underwent abdominoperineal resection. En-bloc resection of entire pelvic lymph nodes with the bilateral internal iliac arteries and veins was performed for all patients. Resected organs included the rectum in 20 cases, the urinary bladder in 39, the uterus and vagina in 8, the external genitalia in 2, the obturator internis muscle in 12, the gluteus maximus muscle in 5, and the small intestine in 7. Urinary diversions were an ileal conduit in 37 patients and a ureterocutaneostomy in 2. Three patients underwent complete resection of one, one, and three synchronous liver metastases. In addition, one patient underwent complete resection of four peritoneal metastases.

Follow-Up

One patient returned to Indonesia and was lost to follow-up. The other 43 were followed up completely, with a median follow-up time for live patients of 4.7 years (range, 1.2–15.8 years). They were examined with abdominal and pelvic CT, chest roentgenogram or CT, and carcinoembryonic antigen (CEA) measurement every 4 months for 0 to 1 years, every 6 months for 2 to 4 years, and annually for 5 to 10 years.

Statistical Analysis

Survival, disease-free survival, and local disease–free survival distributions were estimated by using the Kaplan-Meier product-limit method. Univariate comparisons of survival were made by using the log-rank test, and multivariate analysis was performed by using the Cox regression model with the forward stepwise method (likelihood ratio). All variables were dichotomized for analysis. Differences in proportions were analyzed by Fisher's exact test and by multivariate analysis with the logistic regression model and the forward stepwise method (likelihood ratio). All statistical analyses were performed with SPSS for Windows, version 10.0J (SPSS-Japan Inc., Tokyo, Japan). All P values were two sided, and a P value of $< .05$ was considered to be statistically significant.

RESULTS

Pathologic Findings

Histological diagnoses of the PPR cases are listed in Table 1. The bone marrow or periosteum of the sacrum was histologically involved in 17 patients. The remaining 27 had no sacral invasion, but dense fibrotic tissues adhered extensively to the sacrum, and cancer cells were found within them. Of 13 patients with pelvic lymph node involvement, 12 had intrapelvic metastases alone, and 1 had both intrapelvic and extrapelvic metastases. Eight patients had distant metastasis, including liver metastasis in three, lung metastasis in three, peritoneal metastasis in one, and distant lymph node metastasis in one.

Resection margins were microscopically negative in 24 patients, microscopically positive in 13, macroscopically positive in 3, and grossly residual in 4 (lung, n = 2; lung and local, n = 1; lymph node, n = 1; Table 1). The sites of macroscopic positive margins included cut ends of the sacrum and/or presacral connective tissue in two, cut ends of the sacral nerves and the external iliac artery in one, and the lateral pelvic sidewall in one. The major artery was involved only in one patient with prior extended lateral pelvic lymph node dissection. The sites of microscopic positive margins included the cut end of the sacrum in two, the cut end of the presacral connective tissue in three, the cut ends of the sacrospinous ligament and sacrotuberous ligament in one, the cut ends of the sacrospinous ligament and obturator internis muscle in one, the cut end of the obturator lymph node in one, and the cut ends of the sacral nerves in one.

Macroscopic growth patterns were based on macroscopic views of sections of resected specimens and were classified as solitary expanding growth, multiple expanding growth, and diffuse infiltrating growth (Fig. 1; Table 1). Expanding growth featured smooth

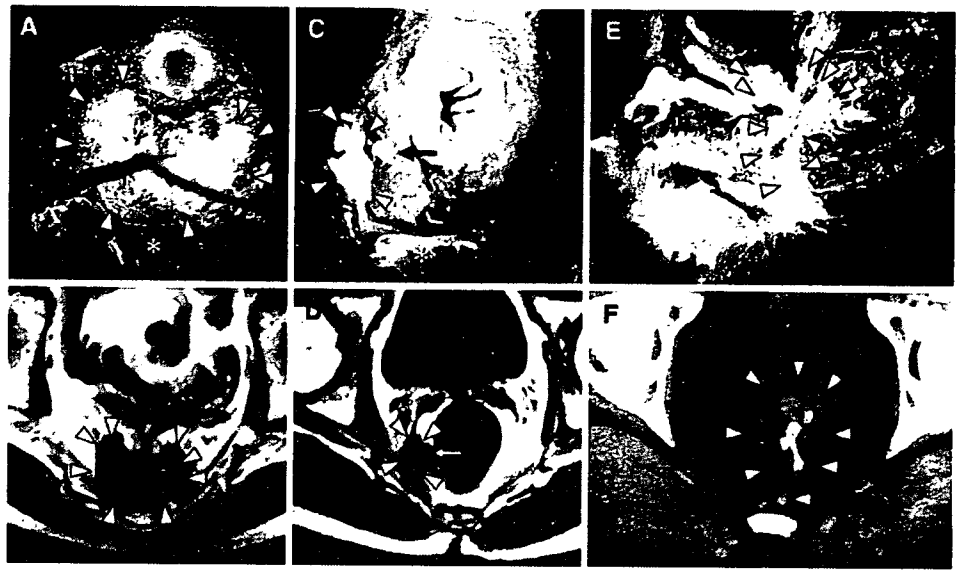

FIG. 1. (A) A section after abdominal sacral resection for posterior pelvic recurrence of rectal carcinoma. This tumor was macroscopically classified as solitary expanding growth. (B) Corresponding magnetic resonance image of (A). (C) A section of tumor macroscopically classified as multiple expanding growth. (D) Corresponding magnetic resonance image of (C). (E) A section of tumor macroscopically classified as diffuse infiltrating growth. (F) Corresponding computed tomography of (E). Arrowheads, main tumor; arrow, satellite tumor. *Sacrum.

and clear margins. Any tumors showing irregular or obscure margins were therefore classified into the diffuse infiltrating category.

Morbidity and Mortality

The median operating time was 751 minutes (range, 263–1377 minutes). The median blood loss was 3208 mL (range, 856–26160 mL), and all of the patients underwent transfusion. Of the 27 patients with postoperative complications (morbidity, 61%), 10 (23%) had major complications that necessitated surgical interventions or resulted in hospital death, and 17 (38%) had minor complications that could be managed conservatively (Table 2). The number of complications per patient was as follows: 4 in 1 patient, 3 in 5 patients, 2 in 10 patients, and 1 in 11 patients. One patient who had pelvic sepsis, residual tumor regrowth, bowel obstruction, and renal failure died on the 66th postoperative day (mortality, 2%).

Eleven (65%) of 17 patients who had received adjuvant or previous radiation had postoperative complications, compared with 16 (59%) of 27 who had not received radiation ($P = .76$). In contrast, 7 (41%) of 17 with adjuvant or previous radiation experienced major complications, compared with 3 (11%) of 27 without irradiation ($P = .03$). The median hospital stay was 38 days (range, 22–316 days).

TABLE 2. *Complications*

Complication	No. Patients
Major complications	
Pelvic sepsis	8
Bowel obstruction	3
Intestinal fistula	2
Ureteroileostomy leakage	2
Ureterocutaneostomy stenosis	1
Ileal conduit necrosis	1
Renal failure	1
Uncontrollable bleeding	1
Postoperative bleeding	1
Tracheal stenosis	1
Minor complications	
Wound dehiscence/infection	6
Bowel obstruction	12
Urinary tract infection	10
Ureteroileostomy stenosis	1
Neurogenic bladder	2

Survival

The median survival for all the patients undergoing ASR was 2.3 years (range, .1–15.8 years). The estimated overall 1-, 3-, and 5-year survival rates were 90%, 47%, and 34%, respectively, including one hospital death (Fig. 2). Of the 15 patients who survived >4 years, 9 were disease free, and 5 survived >8 years. The disease-free 1-, 3-, and 5-year survival rates were 44%, 26%, and 24%, respectively. The local disease–free 1-, 3-, and 5-year survival rates were 63%, 47%, and 47%, respectively (Fig. 2).

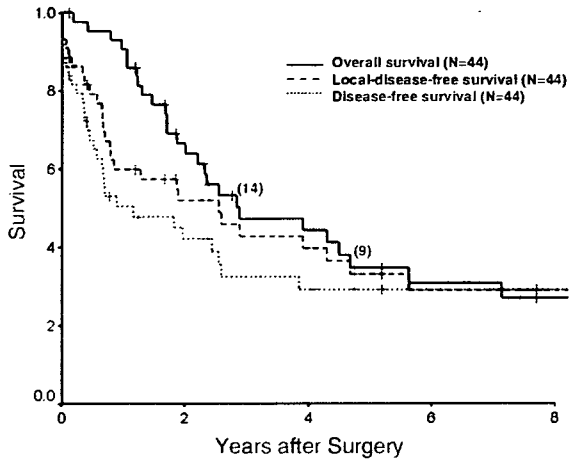

FIG. 2. Overall, disease-free, and local disease–free survival distributions for the 44 patients undergoing abdominal sacral resection for posterior pelvic recurrence of rectal carcinoma. The numbers in parentheses for the overall survival curve indicate the patients alive at 3 and 5 years.

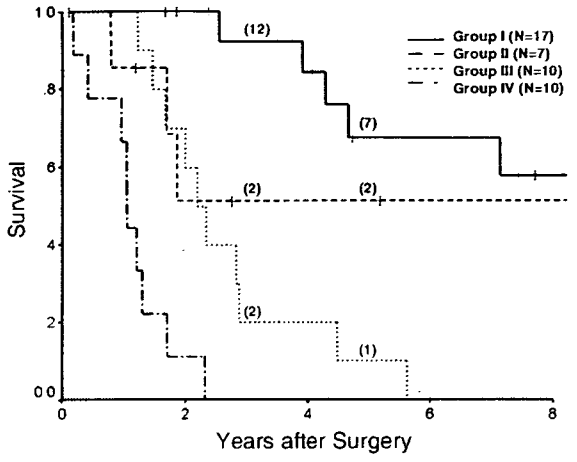

FIG. 3. Overall survival curves for group I (microscopic negative margin and local disease–free interval [LDFI] of >12 months), group II (microscopic negative margin and LDFI <12 months), group III (positive margin and LDFI >12 months), and group IV (positive margin and LDFI <12 months). The numbers in parentheses for each curve indicate the patients alive at 3 and 5 years.

Prognostic Factors

Results of univariate analysis of prognostic factors are summarized in Table 1. The overall survival of the patients with microscopic positive resection margins was significantly worse than that of those with microscopic negative margins ($P < .0001$) but was not significantly better than that of those with macroscopic positive margins or macroscopic residual tumor ($P = .11$). Patients with macroscopic positive margins or macroscopic residual tumor did not survive >2.3 years.

The survival of patients with buttock pain was significantly worse than that of those without pain or with perineal pain ($P = .043$) and was significantly better than that of those with thigh or leg pain ($P = .0046$). The latter died within 1.2 years.

Of the eight patients with distant metastasis, two undergoing resection of solitary liver metastasis were alive and disease free for 7.6 and 2.7 years, one undergoing resection of three liver metastases died of disease at 1.3 years, one undergoing resection of four peritoneal metastases was alive with disease at 1.1 years, three with one or two lung metastases died of disease at 2.3, 2.0, and 1.6 years, and one with paraaortic lymph node metastasis died at 1.7 years.

The univariate analysis of the 15 variables (Table 1), when dichotomized, showed a positive resection margin, pain extending to the buttock or further, multiple growths or diffuse infiltrating growth, LDFI of <12 months, a preoperative CEA level >10 ng/mL, and primary cancer stage IV to be associated with significantly worse survival. The other nine factors did not show any significant association with outcome.

The multivariate analysis of the 15 dichotomized variables revealed that only a positive resection margin (hazard ratio, 10 [95% confidence interval, 3.8–28]; $P < .001$), an LDFI of <12 months (4.2 [1.8–9.8]; $P = .001$), and pain radiating to the buttock or further (4.2 [1.6–11]; $P = .004$) were independently associated with worse survival.

When the most significant independent factors were considered together, the 5-year overall survival rates of the 17 patients with microscopic negative margins and an LDFI >12 months (group I), the 7 with microscopic negative margins and an LDFI <12 months (group II), the 10 with positive margins and an LDFI >12 months (group III), and the 10 with positive margins and an LDFI <12 months (group IV) were 67%, 51%, 10%, and 0%, respectively (Fig. 3). There were significant survival differences between group I and group III ($P < .0001$), group III and group IV ($P = .0014$), and group II and group IV ($P = .01$). Group IV patients did not survive >2.3 years.

Risk Factors for a Positive Resection Margin

To clarify the risk factors for a positive resection margin, the most significant prognostic factor on multivariate analysis, univariate and multivariate analyses were conducted. Three patients who under-