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Ⅲ. 研究成果の刊行物・別刷

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Genetic Variations and Haplotype Structures of the *ABCB1* Gene in a Japanese Population: An Expanded Haplotype Block Covering the Distal Promoter Region, and Associated Ethnic Differences

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Summary

As functional *ABCB1* haplotypes were recently reported in the promoter region of the gene, we resequenced the *ABCB1* distal promoter region, along with other regions (the enhancer and proximal promoter regions, and all 28 exons), in a total of 533 Japanese subjects. Linkage disequilibrium (LD) analysis based on 92 genetic variations revealed 4 LD blocks with the same make up as previously described (Blocks – 1, 1, 2 and 3), except that Block 1 was expanded to include the distal promoter region, and that a new linkage between polymorphisms – 1789G>A in the distal promoter region and IVS5 + 123A>G in intron 5 was identified. We re-assigned Block 1 haplotypes, and added novel haplotypes to the other 3 blocks. The reported promoter haplotypes were further classified into several types according to tagging variations within Block 1 coding or intronic regions. Our current data reconfirm the haplotype profiles of the other three blocks, add more detailed information on functionally-important haplotypes in Block 1 and 2 in the Japanese population, and identified differences in haplotype profiles between ethnic groups. Our updated analysis of *ABCB1* haplotype blocks will assist pharmacogenetic and disease-association studies carried out using Asian subjects.

Keywords: *ABCB1*, P-gp, haplotype

Introduction

The *ABCB1* gene, encoding p-glycoprotein (P-gp)/multidrug resistance protein 1 (MDR1), is located on chromosome 7q21-q31 and consists of 28 exons. P-gp (1280 amino acids), a member of the

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ATP-binding cassette (ABC) transporter superfamily, is a large transmembrane glycoprotein that consists of two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs). P-gp was initially identified as a component of the multidrug resistance phenotype in cancer cells (Riordan *et al.* 1985), but was later found to be widely expressed in normal epithelial cells of tissues such as the liver, intestine, kidneys, and the blood-brain and testis barriers, as well as in lymphocytes (Fojo *et al.* 1987; Cordon-Cardo *et al.* 1989). It is thought that P-gp plays a role in the protection of these tissues against structurally-unrelated toxic xenobiotics, and can modify the oral bioavailability and renal secretion of a variety of drugs (Hoffmann & Kroemer, 2004). Multiple other physiological functions of P-gp have also been suggested in lipid transport (van Helvoort *et al.* 1996), cholesterol metabolism (Debry *et al.* 1997), inhibition of ceramide-induced apoptosis (Liu *et al.* 2001), and the initiation of immune responses by cytokine release (Drach *et al.* 1996). Moreover, reduced P-gp expression has been linked to cancer (Siegmund *et al.* 2002) and other diseases such as Parkinson's disease (Furuno *et al.* 2002) and ulcerative colitis (Schwab *et al.* 2003).

With recent advances in genomics research there has been an increasing number of pharmacogenetic studies focused on the *ABCB1* gene. Hoffmeyer *et al.* (2000) showed that a synonymous 3435C>T mutation in exon 26 was associated with reduced P-gp expression in the duodenum, and increased plasma levels of digoxin following its oral administration in healthy volunteers. Thus, the 3435C>T single nucleotide polymorphism (SNP) has become the focus of much attention. However, reports on the role of this common SNP have been very inconsistent, which suggests that other functional polymorphisms may be linked with 3435C>T (Kim, 2002). Further studies revealed that 3435C>T was closely linked to other common polymorphisms, such as 1236C>T (silent) at exon 12 and 2677G>T (Ala893Ser) at exon 21, and that the combinations of these SNPs (i.e. haplotypes) differed greatly between ethnic groups (Kim *et al.* 2001; Kroetz *et al.* 2003; Tang *et al.* 2002, 2004). While an *in vitro* functional study on the nonsynonymous 2677G>T (Ala893Ser) SNP at exon 21 showed that 2677G>T was associated with enhanced P-gp activity (Kim *et al.* 2001), other stud-

ies found no association (Kimchi-Sarfaty *et al.* 2002; Morita *et al.* 2003; Kroetz *et al.* 2003). One of these latter studies also revealed that another nonsynonymous SNP, 2677G>A (Ala893Thr), had no impact on P-gp function (Morita *et al.* 2003). Yet several clinical studies have shown that the haplotypes 2677T-3435T and 1236T-2677T-3435T are associated with reduced P-gp activity (Johns *et al.* 2002; Kurata *et al.* 2002; Chowbay *et al.* 2003; Wong *et al.* 2005), and that 2677A-bearing subjects exhibit higher P-gp activity (Yi *et al.* 2004). Studies that found no association between these *ABCB1* SNPs and P-gp expression levels (Goto *et al.* 2002), and other conflicting results, have been summarized in recent review articles (Kim, 2002; Ieiri *et al.* 2004).

Recently, *ABCB1* gene promoter region haplotypes were reported by two Japanese research groups, and revealed the existence of functional haplotypes that resulted in altered P-gp expression (Taniguchi *et al.* 2003; Takane *et al.* 2004). In these studies, haplotypes that included -1789G>A alone or in combination with -145C>G were associated with decreased P-gp expression. However, the reported effects of haplotypes carrying -129T>C and two other linked SNPs on P-gp expression were contradictory, showing reduction and enhancement.

From these findings it is clear that the establishment of detailed *ABCB1* gene haplotype profiles specific for each ethnic group is important. We previously conducted haplotype analysis on 145 Japanese subjects by dividing the *ABCB1* gene into 4 blocks, one of which included the proximal promoter region, and revealed that the *2 haplotype in Block 2, which harbours 1236C>T, 2677G>T and 3435C>T, showed a strong association with reduced renal clearance of irinotecan and its metabolites (Sai *et al.* 2003). However, recent findings on the functional distal *ABCB1* promoter region prompted us to identify the extended haplotypes that encompassed the above promoter region in a larger Japanese population.

In this study, we sequenced the distal *ABCB1* gene promoter regions from 533 Japanese subjects. This region covered approximately 2.5 kb upstream from the translational initiation site, adjacent to the previously described Block 1 region. We found that the promoter region SNPs were closely linked with SNPs located over a relatively wide range (up to intron 5) in Block 1, such

Table 1 Additional primers used for sequencing of the *ABCB1* gene promoter region

Primer name	Forward primer (5' to 3')	Primer name	Reverse primer (5' to 3')
First amplification*			
MDR1-1ZF1	CCTGCTCTGTTTTTCACCGT	MDR1-1ZR1	ATTGGTTTCCTCTATGCAGA
Second amplification			
MDR1-P1F	GAGAGGGACTACTGGTTAGC	MDR1-P1R	TGGTCCATCTGGGGTAAATG
MDR1-P2F	AAGGACTGTTGAAAGTAGCA	MDR1-P2R	TTTGAGACGGAGTCTTGCTT
MDR1-P3F	CAGAGATCATAGGCACAAAT	MDR1-P3R	AAACTTCAGACGTGATCA
MDR1-P4F	GAAACATCCTCAGACTATGC	MDR1-P4R	CAGGAGGAATGTTCTGGCTT
Sequencing			
MDR1-P5F	ATTTCTTTGAAGTGCTTGGC	MDR1-P5R	GCCACCACCACTTCTGTCAA
MDR1-P6F	GATCTTTACCTGATGCTCAA	MDR1-P6R	GTGCCATGATCTCTGTTTT
MDR1-P7F	AGCTCACGCCTGTAATCCCT	MDR1-P1R	TGGTCCATCTGGGGTAAATG
MDR1-P4F	GAAACATCCTCAGACTATGC	MDR1-P8R	AGGAAAAGTACGTGCAATCT
MDR1-P9F	ACGTACTTTTCCTCAGTTTG	MDR1-P9R	ACACGTCTTTCAAAGTTCAC

Other primer sets used were as previously reported (Sai *et al.* 2003).

*The same set as previously used for the enhancer and promoter regions.

that it was necessary to re-evaluate the functional significance of Block 1 haplotypes. We also sequenced the same regions as covered by the previous study, including the enhancer region (Geick *et al.* 2001) and all exons and surrounding introns, for an additional 388 subjects. These results allowed us to add novel haplotypes to three other blocks. Lastly, we performed a network analysis on the haplotypes obtained in each block and compared the profile of *ABCB1* haplotypes in Japanese with those of other ethnic groups (Kroetz *et al.* 2003; Takane *et al.* 2004).

Materials and Methods

DNA Samples

All 533 Japanese subjects were patients with either ventricular tachycardia (121 subjects) who were administered an anti-arrhythmic drug (amiodarone) and/or β -blockers, or with various cancers (412 subjects) who were administered an anti-cancer drug (paclitaxel or irinotecan). Genomic DNA was extracted directly from blood leukocytes. This study was approved by the ethical review boards of the National Cardiovascular Center, the National Cancer Center, and the National Institute of Health Sciences. Written informed consent was obtained from all subjects.

DNA Sequencing

Amplification and sequencing of the *ABCB1* gene were performed as previously described (Sai *et al.* 2003), ex-

cept that the region sequenced included the promoter region up to 2.5 kb upstream from the translational initiation site. For the promoter region, PCR amplification was first performed using the previous primer set that covered from 7 kb upstream of the transcription site to exon 3, and then new primer sets were used for the second PCR and sequencing (Table 1). Amplification and sequencing primers for the other regions and the PCR conditions used were the same as previously reported (Sai *et al.* 2003). Genbank NT_007993.14 was used as the reference sequence. Nucleotide positions were based on cDNA sequence as previously described, with the adenine of the translational initiation site at exon 2 numbered as +1. For 5'-flanking variations intron 1 was skipped for numbering nucleotide positions.

Haplotype and Network Analyses

Linkage disequilibrium (LD) analysis was performed using SNPalyze software (Dynacom Co., Yokohama, Japan). According to the LD pattern we divided the *ABCB1* gene into 4 blocks following the previously described block partitioning, except for a changed border between Block 1 and Block 2 (IVS5 + 123A>G was shifted from Block 2 to Block 1). Diploidy configurations (combinations of haplotypes) in each block were inferred by LDSUPPORT software, which determined the posterior probability distribution of diploidy configurations for each subject based on estimated haplotype frequencies (Kitamura *et al.* 2002). As Block 1 was expanded we re-defined the Block 1 haplotypes.

For Block 2 haplotypes the previously defined *8c was deleted due to a shift of IVS5 + 123A>G to Block 1. For the rest of the haplotypes we followed the haplotype nomenclature used in our previous study (Sai *et al.* 2003) and added the newly-identified haplotypes consecutively. In our nomenclature the group of haplotypes without amino acid changes or marker SNPs in Block 2 (1236C>T, 2677G>T/A and 3435>T) was defined as *1, and haplotype groups bearing non-synonymous SNPs or marker SNPs in Block 2 were consecutively numbered as described previously (Sai *et al.* 2003). Novel haplotypes within each haplotype group were designated in descending order of frequency. Haplotypes inferred in only one patient, or ambiguously defined, were described with "?", and some rare variations described as "Others" in Figures 3–5. To allow comparison with previous reports (Taniguchi *et al.* 2003; Takane *et al.* 2004) an additional classification for Block 1 haplotypes was given in Fig. 7, based on marker SNPs of the promoter region (–1789G>A, –1461–1457delCATCC, –371A>G, –145C>G and –129T>C).

Network analysis of haplotypes was performed to obtain cladograms using Network 4.1.0.9 (www.fluxus-engineering.com). Network calculations were based on algorithms of the reduced median network (for Blocks –1, 1 and 3) or the median joining network (for Block 2). Haplotypes inferred in only one patient were omitted from the network analysis due to their low predictability.

Results

Additional Genetic Variations

In this study we sequenced the distal promoter region covering approximately 2.5 kb upstream of the translational initiation site in exon 2 in 533 Japanese subjects. We also re-sequenced the enhancer region, and all 28 exons and surrounding regions (the same regions that were sequenced in the previous paper), in an additional 388 subjects. A total of 92 genetic variations were detected in the entire region sequenced in this study. All of the allelic frequencies were in Hardy-Weinberg equilibrium. Since we did not find any apparent differences

in SNP frequencies between the two disease types ($P \geq 0.2233$; Fisher's exact test), the data from all subjects were analyzed as one group.

In addition to the variations reported in our previous study we detected 44 further variations, including 35 novel variations, as listed in Table 2. Novel variations included 8 nonsynonymous substitutions: 49T>C(F17L), 144G>T(K48N), 304G>C(G102R), 1342G>A(E448K), 1804G>A(D602N), 2359C>T(R787W), 2719G>A(V907I) and 3043A>G(T1015A); and 2 synonymous substitutions: 354C>T(Y118Y) and 447A>G(K149K); with frequencies ranging from 0.001 to 0.005. Other novel variations in the 5'-flanking region were 11 nucleotide substitutions and one deletion, while in the intronic regions there were 11 nucleotide substitutions, one deletion, and one insertion (Table 2).

The highly polymorphic variations 1236C>T, 2677G>T, 2677G>A, and 3435C>T were detected at frequencies of 0.572, 0.410, 0.183, and 0.440, respectively, which was consistent with our previous observations (Sai *et al.* 2003). In the newly-sequenced promoter region the reported polymorphic variations –1847T>C, –1789G>A, –1461–1457delCATCC, and –1347T>C were found at frequencies of 0.084, 0.204, 0.030, and 0.084, respectively, which were comparable with frequencies in Japanese in previous reports (Taniguchi *et al.* 2003; Takane *et al.* 2004).

LD analysis was performed using the 92 detected genetic variations, and pairwise rho square (r^2) values for the representative 46 polymorphisms (alleles detected in 5 or more chromosomes), and the results are shown in Fig. 1. With the additional distal promoter region sequence close linkage relationships were observed between –1847T>C, –1347T>C, –371A>G, –129T>C, IVS3 + 36C>T and IVS5 + 76T>G. A close linkage was also detected between –1789G>A in the promoter region and IVS5 + 123A>G in intron 5 (formerly classified as Block 2). Based on these linkage relationships we changed the previous border between Block 1 and Block 2, such that IVS5 + 123A>G was now classified as part of Block 1. The other linkage profiles were the same as previously described, confirming the previous partitioning between Blocks 2 and 3. Similarly, the enhancer region at around 7 kb

Table 2 Additional ABCB1 variations detected in Japanese

Block	SNP ID			Position		Nucleotide change	Amino acid change	Frequency
	This study ^a	Reference	Site	NT_007933.14	cDNA-based			
Block - 1	MPJ6_AB1078	(novel)	5'-Flanking	12472468..12472461	- 8128..- 8121	G T A A A G T C A G A T C T A A C C A A / - C T G T T C A T T G G T		0.002
	MPJ6_AB1079	(novel)	5'-Flanking	12466729	- 2389	C T C C C A T A G A T A C / T A T A T A G A A C A G A		0.001
	MPJ6_AB1080	b)	5'-Flanking	12466680	- 2340	A T G T T G T C A G A G I / C A T A G A C A A G T T G		0.001
	MPJ6_AB1081	(novel)	5'-Flanking	12466659	- 2319	G T T G G T G A A A T G G / T C T A C A T G A G A G C		0.001
	MPJ6_AB1072	b,c)	5'-Flanking	12466187	- 1847	G T T T A G G G A G G G T / C T T A A G G C C A T T C		0.084
	MPJ6_AB1073	rs12720464 ^d	5'-Flanking	12466129	- 1789	A A T G A A A G G T G A G / A A T A A A G C A A C A A		0.204
	MPJ6_AB1082	(novel)	5'-Flanking	12466065	- 1725	A A G A T T A A A A A C G / A C A T G T A A T G A A G		0.001
	MPJ6_AB1083	(novel)	5'-Flanking	12465983	- 1643	C A G T G A A C A A T G C / I T G T A C A C T T G C A		0.001
	MPJ6_AB1084	(novel)	5'-Flanking	12465806	- 1466	G G T C A G G A G A T C A / G A G A C C A T C C T G G		0.002
	MPJ6_AB1085	c)	5'-Flanking	12465801..12465797	- 1461..- 1457	G G A G A T C A A G A C C A I C C / - T G G C T A A C A C A G		0.030
Block 1	MPJ6_AB1074	b,c)	5'-Flanking	12465687	- 1347	G C A G G A G A A T G G T / C G T G A A C C C G G G A		0.084
	MPJ6_AB1086	(novel)	5'-Flanking	12465619	- 1279	C C T G G C C G A C A A A / G G C A A G A C T C C G T		0.004
	MPJ6_AB1075	b,c)	5'-Flanking	12465494	- 1154	A G A A A A A A T T A T / C G G C T T T G A A G T A		0.001
	MPJ6_AB1087	(novel)	5'-Flanking	12465444	- 1104	A T C C T C A G A C T A T / C G C A G T A A A A A A C		0.001
	MPJ6_AB1088	(novel)	5'-Flanking	12465421	- 1081	A C A A A G T G A T T T T / C C T T C T T C T A A A C		0.002
	MPJ6_AB1089	(novel)	5'-Flanking	12465405	- 1065	C T T C T A A C T T A T / C G C A A T A A A C T G A		0.001
	MPJ6_AB1090	(novel)	5'-Flanking	12465326	- 986	T C C T C T A T G T T C A / G T A A G A A G T A A G A		0.001
	MPJ6_AB1091	(novel)	5'-Flanking	12464967	- 627	T T A T C A T C A A A T A / G A A G G A T G A A C A G		0.002
	MPJ6_AB1092	(novel)	Exon 2	12463728	49	A A G A A G A A C T T T T T / C T T A A A C T G A A C A	F17L	0.001
	MPJ6_AB1093	(novel)	Exon 4	12449246	144	T T G G C T T G A C A A G / T T T G T A T A T G G T G	K48N	0.001
MPJ6_AB1094	(novel)	Exon 5	12433798	304	A T C A A T G A T A C A G / C G G T T C T T C A T G A	G102R	0.005	
MPJ6_AB1095	(novel)	Exon 6	12430553	354	T G C C T A T A T A T A C / T A G T G G A A T T G G T	Y118Y	0.001	
MPJ6_AB1096	(novel)	Exon 6	12430460	447	C A A A A T T A G A A A A / G C A G T T T T T T C A T	K149K	0.002	
MPJ6_AB1097	(novel)	Exon 12	12413771	1342	T A T G A C C C C C A C A G / A A G G G G A T G G T G A	E448K	0.001	

Table 2 Continued.

Block	SNP ID		Reference	Site	Position		Nucleotide change	Amino acid change	Frequency
	This study ^a	NT_007933.14			cDNA-based				
Block 2	MPJ6_AB1052	12413746	Intron 12	IVS12 +17	GATGACCCATGCG/AGCTAGACCCCTG			0.006	
	MPJ6_AB1098	12413720	Intron 12	IVS12 +43	GGTGATCAGCAGT/GCACATTGCACAT			0.001	
	MPJ6_AB1099	12413353	Intron 13	IVS13 +90	CTACTATAAATCG/AGAAGAAGGGAAA			0.001	
	MPJ6_AB1100	12409538	Exon 15	1804	ATCGCTGGTTTCG/AATGATGGAGTCA		D602N	0.002	
	MPJ6_AB1101	12408686	Intron 15	IVS15 -95	GTTACTAAACAAA/GTTGCTGTTTTCC			0.001	
	MPJ6_AB1065	12408363	Intron 16	IVS16 +52	CTGTGGTCCCTA/CGTTGGTGGGCT			0.003	
	MPJ6_AB1102	12407939	Intron 16	IVS16 -72	TCCITTAATAAT/ATTTGTGCGTATG			0.001	
	MPJ6_AB1103	12404862	Intron 18	IVS18 +87	AGTGTAAATTGGCC/TTTTAGTAGAAC			0.001	
	MPJ6_AB1104	12402898	Exon 19	2359	ATCCTCACCAAGC/TGGCTCCGATACA		R787W	0.001	
	MPJ6_AB1105	12400221	Intron 19	IVS19 -88	GGGTATAAGTAT/CAAAACAAAACCTGA			0.001	
	MPJ6_AB1106	12395242	Intron 20	IVS20 -153	TTCITTACTGTAGA/GAACTCAATAAAC			0.001	
	MPJ6_AB1107	12395172	Intron 20	IVS20 -83	GAATAGTCTCA/GTGAAGGTGAGTT			0.001	
	MPJ6_AB1108	12384544_12384541	Intron 21	IVS21 -73--76	TTATTTTCATTAGTCT/-GTTTTATAGAAT			0.003	
	MPJ6_AB1067	12384435	Exon 22	2719	AACITCCGAACCG/ATTGTTTCTTTGA		V907I	0.002	
	MPJ6_AB1109	12384359	Intron 22	IVS22 +9	ACAGGTAATAAACCT/TGCTGAAGAGTGG			0.001	
	MPJ6_AB1076	12380229	Exon 24	2956	GTCTTTGGTGCCA/GTGGCCGTGGGGC		M986V	0.001	
MPJ6_AB1110	12380142	Exon 24	3043	ATCATTGAAAAAA/GCCCTTTTGATG		T1015A	0.001		
Block 3	MPJ6_AB1111	12372831_12372834	Intron 26	IVS26 +33_36	ACAGCCTGGGAG/-CAITGGGCAGCCTCTC			0.001	
	MPJ6_AB1112	12369713	Intron 26	IVS26 -78	ATATAGAATCGTC/GTATCCTACTTTC			0.001	
	MPJ6_AB1077	rs2235051 ^d	Exon 28	3747	GTTTCAGAATGGC/GAGAGTCAAGGAG		G1249G	0.002	

All ABCB1 genetic variations in the above list and detected in the previous study (Sai et al. 2003) were used for the haplotype analysis in this study.

^aSNP ID assigned by our project team (MPJ-6).

^bTaniguchi et al. 2003.

^cTakane et al. 2004.

^dNCBI dbSNP

^eIroda et al. 2002.

^fTanabe et al. 2001.

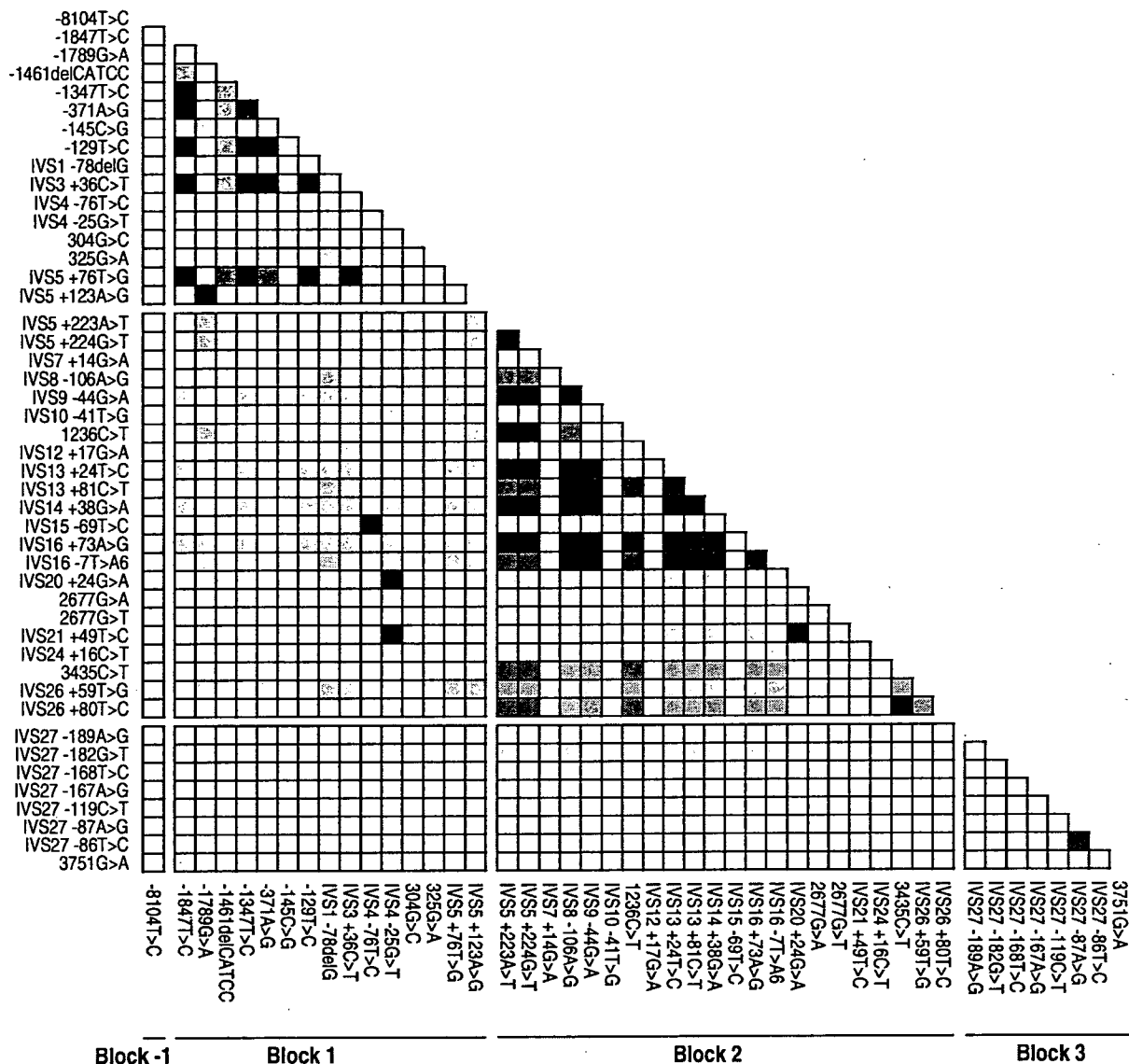


Figure 1 Linkage disequilibrium (LD) analysis of the *ABCB1* gene. Pairwise LD (r^2 values) of the polymorphisms detected in 5 or more chromosomes is shown as a 10-graded blue colour.

upstream of the transcriptional start site was assigned as Block -1 as described previously.

Haplotype Analysis

We estimated the diplotype configurations (haplotype combinations) of all 4 blocks using LDSUPPORT software. DiploTYPE configurations were obtained at probabilities over 0.9 for 100%, 92%, 95%, and 98% of the subjects for Blocks -1, 1, 2, and 3, respectively.

In Block -1, one novel haplotype, *1d, was identified. Thus Block 1 contained four *1 haplotypes (Fig. 2). The most common haplotype was *1a with a frequency of 0.988.

For Block 1 five haplotype groups consisting of 39 haplotypes were newly assigned. Of the 35 haplotypes in the *1 group, 10 haplotypes were ambiguous and were included as "Others" in Fig. 3. Haplotype groups *2 to *5 were defined by the nonsynonymous SNPs 325G>A(E109K) (*2), 304G>C(G102R) (*3),

49T>C(F17L) (*4) and 144G>T(K48N) (*5). The most frequent haplotype was *1a at a frequency of 0.541, followed by *1b (-1789G>A and IVS5 + 123A>G), *1c (IVS1 - 78delG), and *1d (IVS4 - 25G>T) at frequencies of 0.098, 0.079, and 0.041, respectively. The nonsynonymous *2 and *3 groups occurred at frequencies of 0.017 and 0.005, respectively.

Site		5'-Flanking				
Position	-8128 -8121	-8104	-7970			
Nucleotide change	del CTAA CCAA	T>C	C>T			
Amino acid change				N	Frequency	
*1	*1a			1053	0.988	
	*1b			2	0.002	
	*1c			9	0.008	
	*1d			2	0.002	

Figure 2 ABCB1 haplotypes in Block -1 for 533 Japanese subjects. The haplotype nomenclature followed the definitions used in our previous study (Sai et al. 2003). Newly identified haplotypes were consecutively named as shown in boldface. N: number of chromosomes analyzed.

In Block 2 15 haplotype groups consisting of 61 haplotypes were inferred, including 38 newly-defined haplotypes. Of the 61 haplotypes 24 were detected in only one patient or ambiguously inferred. Ambiguous haplotypes within each group (groups *1, *8, and *10) were indicated as "Others" or "?" in Fig. 4. The most frequent haplotype was the *2 group at a frequency of 0.386, which harboured 1236C>T (exon 12), 2677G>T(A893S) (exon 21) and 3435C>T (exon 26). Groups *1, *10 [2677G>A(A893T)] and *8 (1236C>T) were found at frequencies of 0.216, 0.174 and 0.141, respectively. Other minor haplotype groups were *6 (3435C>T), *9 [1236C>T and 2677G>T(A893S)], *4 (1236C>T and 3435C>T) and *11 [1236C>T and 2677G>A(A893S)] at frequencies of 0.034, 0.020, 0.016, and 0.005, respectively. All these frequencies were comparable with our previous findings (Sai et al. 2003). Novel haplotype groups bearing amino acid substitutions were assigned as *12 [1804G>A (D602N)], *13 [2719G>A (V907I)], *14 [1342G>A (E448K)], *15 [2956A>G (M986V)], *16 [3043A>G (T1015A)], and *17 [2359C>T(R787W)],

Site		5'-UTR															Ex. 1		Int. 1		Ex. 2		Int. 3		Ex. 4		Int. 4		Ex. 5		Int. 5			
Position	-2340	-2319	-1847	-1789	-1466	-1461 -1457	-1347	-1279	-1081	-1065	-986	-371	-145	-129	IVS1 -78	49	IVS3 +36	144	IVS4 -76	IVS4 +25	304	325	IVS5 +76	IVS5 +123										
Nucleotide change	T>C	G>T	T>C	G>A	A>G	delCA TCC	T>C	A>G	T>C	T>C	A>G	A>G	C>G	T>C	del G	T>C	C>T	G>T	T>C	G>T	G>C	G>A	T>G	A>G										
Amino acid change																F17L	K48N	G102R E109K						N	Frequency									
*1a																														1053	0.541			
*1b																														2	0.098			
*1c																														9	0.079			
*1d																														2	0.041			
*1e																														1	0.029			
*1f																														1	0.028			
*1g																														1	0.028			
*1h																														1	0.022			
*1i																														1	0.017			
*1j																														1	0.016			
*1k																														1	0.015			
*1l																														1	0.012			
*1m																														1	0.010			
*1n																														1	0.010			
*1o																														1	0.004			
*1p																														1	0.003			
*1q																														1	0.002			
*1r																														1	0.002			
*1s																														1	0.002			
*1t																														1	0.002			
*1u																														1	0.002			
*1v																														1	0.001			
*1w																														1	0.001			
*1x																														1	0.001			
*1y																														1	0.001			
Others																														1	0.009			
*2	*2a																													1	0.017			
*3	*3a																													1	0.005			
*4	*4a?																													1	0.001			
*5	*5a?																													1	0.001			

Figure 3 ABCB1 haplotypes in Block 1 for 533 Japanese subjects. Block 1 haplotypes were newly defined due to the change of the Block 1 border. Rare and ambiguous haplotypes (n = 1) are shown with "?". Haplotypes assigned in only one patient or ambiguously inferred are shown as "Others". Sites for nonsynonymous substitutions are indicated by their group-name numbers. N: number of chromosomes analyzed.

Site	Int. 5	Ex. 6	Int. 6	Int. 7	Int. 8	Int. 9	Int. 10	Ex. 12	Int. 12	Int. 13	Int. 14	Ex. 15	Int. 15	Int. 16	Int. 16	Int. 18	Int. 18	Int. 18	Int. 19	Int. 20	Ex. 21	Int. 21	Ex. 22	Ex. 24	Int. 24	Ex. 28		
Position	IVS5 +223	IVS6 +47	IVS6 -109	IVS7 +14	IVS8 -100	IVS9 -44	IVS10 -41	IVS10 +126	IVS12 +142	IVS12 +17	IVS13 +81	IVS14 +38	IVS14 -84	IVS15 +52	IVS16 +73	IVS16 -78	IVS16 +87	IVS18 -35	IVS18 +286	IVS20 +24	IVS20 -153	IVS21 +87	IVS21 -78	IVS21 -78	IVS24 +18	IVS28 +59		
Nucleotide change	A>T	A>G	G>T	G>A	A>G	G>A	T>G	G>T	G>A	G>A	T>C	G>A	A>G	T>C	A>C	T>A	G>T	G>C	T>C	G>A	A>G	G>A	T>C	A>G	A>G	C>T	T>G	
Amino acid change	K16K							E44K				D50N							R79W		A87S	G>T	A87S	V89I	M88V	I101A		
N	117	47	27	10	6	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	5
Frequency	0.110	0.044	0.025	0.009	0.006	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
1																												
2																												
4																												
6																												
8																												
9																												
10																												
11																												
12																												
13																												
14																												
15																												
16																												
17																												
18																												

Figure 4 ABCB1 haplotypes in Block 2 for 533 Japanese subjects. Haplotype nomenclature followed the definitions used in our previous study (Sai et al. 2003) except for deletion of *8c. Newly identified haplotypes were consecutively named as shown in boldface. Rare and ambiguous haplotypes (n = 1) are shown with "?". Haplotypes assigned in only one patient or ambiguously inferred are shown as "Others". Marker sites for the *2 to *18 groups are indicated by their group-name numbers. N: number of chromosomes analyzed.

with frequencies that ranged from 0.002 for *12 and *13, to 0.001 for *14 to *17. Another new haplotype was defined as *18 based on the simultaneous presence of 2677G>A (A893T) and 3435C>T, with a frequency of 0.001. It was also noted that *1f in Block 2 was completely linked with *1d (IVS4 – 25G>T) in Block 1.

In Block 3 three haplotype groups consisting of 21 haplotypes were inferred, including four new haplotypes. Of the 21 haplotypes three were ambiguously inferred and included in "Others" in Fig. 5. The most frequent haplotype was *1a with a frequency of 0.753, followed by *1b (0.176). As observed previously, the rare haplotype groups *2 [3751G>A (V1251I)] and *3 [3587T>G (I1196S)] were observed at frequencies of 0.014 and 0.001, respectively.

We also analyzed the diplotype combinations for all 4 blocks (i.e. the whole gene) for all 533 subjects. The combination patterns were highly diverse with a total of 353 diplotype combinations observed. The frequencies for the majority of diplotypes were less than 0.01. The 10 major combinations are listed in Table 3; all combinations were made up of the major haplotypes in each block.

Network Analysis and Nucleotide Diversity

We performed a network analysis of the haplotypes in each block to obtain cladograms based on the sites and numbers of mutational events. For Block – 1 the rare haplotypes, *1b to *1d, appeared to be derived from the major haplotype: *1a (Fig. 6a). For Block 1 most of the minor haplotypes were connected to one of the major haplotypes *1a or *1b. However, *1e, *1g, *1h, and *1p were shown to be distant from the above haplotypes (Fig. 6b). Haplotype groups including *1b and the closely related haplotypes *1f, *1i, *1k, *1j, *1m, *1L, and *1q were characterized by the presence of – 1789G>A. Of these haplotypes the *1k and *1j subgroups were characterized by the additional SNP – 371A>G, while the subgroups *1m, *1L and *1q contained the SNP – 145C>G. The separate subgroup that consisted of *1e, *1g, *1h and *1p contained

Site	Int. 26		Int. 27										Ex. 28		N	Frequency	
	Position	IVS26 -78	3587	IVS27 +63	IVS27 -189	IVS27 -182	IVS27 -172	IVS27 -168	IVS27 -167	IVS27 -119	IVS27 -87	IVS27 -86	IVS27 -80	3747			3751
Nucleotide change	C>G	T>G	C>G	A>G	G>T	G>A	T>C	A>G	C>T	A>G	T>C	ins C	C>G	G>A			
Amino acid change		I1196S												G1249G	V1251I		
*1	*1a															803	0.753
	*1b															188	0.176
	*1c															27	0.025
	*1d															5	0.005
	*1f															5	0.005
	*1h															4	0.004
	*1j															4	0.004
	*1e															3	0.003
	*1L															2	0.002
	*1g															1	0.001
	*1i															1	0.001
	*1k															1	0.001
	*1m															1	0.001
	*1o															1	0.001
*1r															1	0.001	
Others															3	0.003	
*2	*2a														2	9	0.008
	*2b														2	6	0.006
*3	*3a														3	1	0.001

Figure 5 ABCB1 haplotypes in Block 3 for 533 Japanese subjects. Haplotype nomenclature followed the definitions used in our previous study (Sai et al. 2003). Newly identified haplotypes were consecutively named as shown in boldface. Haplotypes assigned in only one patient or ambiguously inferred are shown as "Others". Sites for nonsynonymous substitutions are indicated by their group-name numbers. N: number of chromosomes analyzed.

the three linked SNPs, $-1847T>C$, $-1347T>C$ and $-129T>C$. This network profile supported the previous classification of promoter region haplotypes by Takane *et al.* (2004), as indicated by the dashed lines. However, our current study revealed the presence of additional subtypes. Detailed comparisons between our Block 1 haplotypes and previously described promoter region haplotypes are described in the next section.

The Block 2 cladogram showed that there were four major haplotypes, *2d, *10a, *1e and *8a, and that most of the minor haplotypes appeared to be derived from *1e, *2d or *8a (Fig. 6c). Network analysis showed that the *2 and *8 haplotypes, which share the SNP 1236C>T, were distant from *1e and *10a, and that *10a and the adjacent *13a that both have 2677G>A without the common polymorphisms 1236C>T, 2677G>T and 3435C>T, are relatively closely related to the *1 group. The *8 group bearing 1236C>T was highly diverse and included many haplotypes. The *4 (1236C>T and 3435C>T) and *9 (1236C>T and 2677G>T) groups were related to *8 and *2. This network profile supported the previous classification of Block 2 haplotypes based on common polymorphisms. For the *6 group, containing 3435C>T, *6a and *6b were distantly related to *1 haplotypes, which suggested that different mutational and/or recombinational events were responsible for these haplotypes.

For Block 3, *1b and the other minor *1 haplotypes were related to the major *1a haplotype, while the *2 group (*2a and *2b) with nonsynonymous SNPs appeared to be derived from either *1a or *1b (Fig. 6d).

Comparison of Block 1 Haplotypes with Reported Promoter Haplotypes

To compare our Block 1 haplotype structures with the previously reported promoter region haplotypes (Taniguchi *et al.* 2003; Takane *et al.* 2004), we classified Block 1 haplotypes into 6 subgroups (A, B, E, G, J and L) based on the marker sites in the reported haplotypes and the network analysis performed in our present study. The summary of this comparison is shown in Fig. 7.

Haplotypes that did not harbour any of the previous markers were classified into subgroup A, in which *1a was the major haplotype. The B subgroup, which included *1b, was defined as haplotypes that contained $-1789G>A$. Haplotypes that contained addi-

tional SNPs $-371A>G$ or $-145C>G$ were classified into subgroups J (including *1j) or L (including *1L), respectively. Subgroup G was defined as those haplotypes that contained the three linked variations ($-1847T>C$, $-1347T>C$ and $-129T>C$) in which *1g was the major haplotype, and subgroup E (including *1e) was defined as those haplotypes with the three linked variations plus $-1461_{-}1457\text{delCATCC}$.

As indicated by the cladograms in the previous section, our study revealed that subgroup A, previously classified as wild-type, could be further classified into six types: the major *1a type without any marker variation and five other types with either IVS1 -78delG (*1c), IVS4 $-25G>T$ (*1d), 325G>A(E109K) (*2a), IVS5 $+123A>G$ (*1n), or 304 G>C(G102R) (*3a). Each of the B, J, and L subgroups that shared $-1789G>A$ were further divided into two types based on the presence of IVS5 $+123A>G$. Subgroup G, with the three reported marker SNPs, was also linked to IVS3 $+36C>T$, and this subgroup was further characterized by the presence of IVS4 $-25G>T$ (*1h and *4a?) or IVS5 $+76T>G$ (*1g). Subgroup E was linked with both IVS3 $+36C>T$ and IVS5 $+76T>G$.

Ethnic Differences

It is well known that there are differences in the frequencies of functionally important haplotypes involving common SNPs (1236C>T, 2677G>T/A and 3435C>T) and promoter region SNPs between different ethnic groups (Kim *et al.* 2001; Kroetz *et al.* 2003; Tang *et al.* 2002, 2004; Takane *et al.* 2004). To characterize these haplotypes in the Japanese population, we compared the frequencies of Block 1 and Block 2 haplotypes that harbour common SNPs with representative reported data from different ethnic groups (Kroetz *et al.* 2003; Takane *et al.* 2004) (Tables 4 and 5). Block 1 haplotype frequencies were generally consistent with those from previous reports for Japanese (Takane *et al.* 2004), except that our study did not detect the reported H7 haplotype that contained $-1154T>C$ alone (Table 4). It has also been suggested that there is much more haplotypic variation in Japanese than in Caucasian populations (Takane *et al.* 2004), and our study supported this.

As for Block 2 haplotypes, the *1 and *2 groups were the common major haplotypes in all the ethnic groups.

Site		5'-Flanking					Ex. 1(5'-UTR)		Int. 1	Ex. 2	Int. 3	Ex. 4	Int. 4	Ex. 5	Int. 5					
Position ^a		-1847	-1789	-1451 -1457	-1347	-371	-145	-129	IVS1 -78	49	IVS3 +36	144	IVS4 -25	304	325	IVS5 +76	IVS6 +123			
Nucleotide change		T>C	G>A	delCAT CC	T>C	A>G	C>G	T>C	del G	T>C	C>T	G>T	G>T	G>C	G>A	T>G	A>G			
Amino acid change									F17L		K48N			G102R	E109K					
Haplotypes		Tagging variations in the previous reports							Additional tagging variations in this study							No. of chromosome	Reported haplotype ¹			
Subgroup	Type																Taniguchi et al (2003)	Takane et al (2004)		
A	*1a type ^b															589				
	*1c type ^c								delG							85				
	*1d type ^d								T							45				
	*2a								delG							18	H1	H1		
	*1n *3a minors ^e								delG							11 5 5				
									(other combinations of SNPs)											
B	*1b type ^f	A														G	126			
	*1f	A														G	30	H2 (low)	H4	
	*5a?	A							5							G	1			
J	*1j	A							G							G	17	H5 (nd)	H5	
	*1k	A							G							G	15			
L	*1L	A							G							G	13			
	*1m	A							G							G	11	H2 (low) or H5 (nd)	H6 (low)	
	minors ^g	A							(G)							G	3			
									(other combinations of SNPs)											
G	*1g	C		C		G		C		T							G	30		
	*1h	C		C		G		C		T							G	23		
	*4a?	C		C		G		C		T							G	1	H3 (low)	H2 (high)
	minors ^h	C		C		G		C		T							G	4		
									(other combinations of SNPs)											
E	*1e type ⁱ	C		del		C		G		T							G	32		H3 (high)

Figure 7 New classification of Block 1 haplotypes and comparison with reported promoter region haplotypes. Genetic variations (allele frequency >0.01) and nonsynonymous variations in Block 1 were sorted according to marker variation, and classified into 6 subgroups (A, B, J, L, G and E).

^aThe positions in other reports were adjusted to the nucleotide numbers used in this study.

^bThe *1a type includes *1a, *1o, *1s, *1t, *1v, *1w, *1x, *1y.

^cThe *1c type includes the *1c haplotype and an ambiguously defined *1 haplotype.

^dThe *1d type includes the *1d haplotype and two ambiguously defined *1 haplotypes.

^e“Minors” include the *1u and *1r haplotypes and one ambiguously defined *1 haplotype.

^fThe *1b type includes the *1b and *1i haplotypes and three ambiguously defined *1 haplotypes.

^g“Minors” include the *1q haplotype and one ambiguously defined *1 haplotype.

^h“Minors” include the *1p haplotype and one ambiguously defined *1 haplotype.

ⁱThe *1e type includes the *1e haplotype and one ambiguously defined *1 haplotype.

^jAltered promoter activity in the reporter gene assay is shown in parenthesis.

nd; not determined.

However, the frequency of the *2 group was much lower than that of the *1 group in Africans. The frequencies of *4 and *8 were higher in Japanese than in Caucasians, and the frequency of the *6 group was higher in Caucasians than in other ethnic groups. The most prominent characteristic of the Japanese population was the high frequency of *10 compared with the other ethnic groups. The variations that characterized *11 to *18 were only detected in our study, probably due to the relatively large number of subjects used. The haplotype distribution in Japanese was similar to that described for Asians, but with slight differences in the frequencies of *6, *8, *9, and *10 reported for a mixed Asian population (Kroetz *et al.* 2003).

Tagging SNPs for ABCB1 Genotyping

For genotyping *ABCB1* in association studies it would be critical to select SNPs for the major haplotypes, including functional ones in Blocks 1 and 2. Table 6 shows the major tagging SNPs for genotyping which are applicable to Japanese and also to other ethnic populations. Genotyping with these SNPs can assign the diploypes of Blocks 1 and 2 in more than 95% of Japanese. The nonsynonymous SNPs in Blocks 1 and 2, and the additional tagging variations in Block 1 obtained in our study (Fig. 7), could be included in the list for evaluation of their functional significance.

Table 4 Ethnic differences in *ABCB1* Block 1 haplotypes

Marker site ^a	This study		Reported data (Takane <i>et al.</i> 2004)		
	Subgroup (see Fig. 7)	Japanese (n = 1066)	Group	Japanese (n = 188)	Caucasian (n = 192)
-1789G>A	A	0.712	H1	0.665	0.964
-1789G>A, -371A>G	B	0.147	H4	0.191	nd
-1789G>A, -145C>G	J	0.031	H5	0.027	nd
-1847T>C ^b	L	0.025	H6	0.032	nd
-1461delCATCC, -371A>G, -1847T>C ^b	G	0.054	H2	0.043	nd
-1154T>C	E	0.030	H3	0.037	nd
-1753delGA		nd	H7	0.005	nd
-1347T>C, -129T>C		nd	H8	nd	0.010
-1085A>G		nd	H9	nd	0.016
			H10	nd	0.010

^aEach reported position was adjusted to the nucleotide numbers used in this study.

^bThis SNP is linked to -1347T>C and -129T>C.

n; 2 × number of subjects.

nd; not detected.

Group	This study	Reported data (Kroetz <i>et al.</i> 2003) ^a		
	Japanese (n = 1066)	Asian (n = 60)	Caucasian (n = 200)	African (n = 200)
*1	0.216	0.216	0.370	0.721
*2	0.386	0.365	0.410	0.075
*3	nd	nd	0.010	0.010
*4	0.016	0.016	0.005	0.090
*6	0.034	0.016	0.120	0.035
*7	nd	nd	0.015	0.005
*8	0.141	0.216	0.010	0.040
*9	0.020	0.082	0.025	0.010
*10	0.174	0.066	0.025	0.005
*11	0.005	nd	nd	nd
*12	0.002	nd	nd	nd
*13	0.002	nd	nd	nd
*14	0.001	nd	nd	nd
*15	0.001	nd	nd	nd
*16	0.001	nd	nd	nd
*17	0.001	nd	nd	nd
*18	0.001	nd	0.01	nd

^aReported haplotypes were re-assigned according to our haplotype nomenclature.

n = 2 × number of subjects.

nd; not detected.

Discussion

Extensive studies of *ABCB1* haplotypes and their functional significance have been conducted, mostly focused on the common SNPs of 1236C>T, 2677G>T/A, and 3435C>T. However, recent association studies on promoter region haplotypes have indicated the importance of haplotypes within this region (Taniguchi

et al. 2003; Takane *et al.* 2004). The results of functional or P-gp expression analyses based on these polymorphisms/haplotypes have not always been consistent, possibly due to the small number of subjects used, different ethnic backgrounds, or insufficient haplotyping over a limited region. In the present study, we have conducted a re-assignment of Block 1 haplotypes by extending the region sequenced to the distal promoter, and

Table 5 Ethnic differences in the *ABCB1* Block 2 haplotypes

Table 6 Major tagging SNPs of *ABCB1* for genotype-phenotype association studies

i) Block 1 haplotypes (subgroups)					
Position	-1847	-1789	-1461_-1457	-371	-145
Nucleotide change	T > C	G > A	delCATCC	A > G	C > G
	a	a	a	a	a
A					
B		A			
J		A		G	
L		A			G
G	C			G	
E	C		del	G	

ii) Block 2 haplotypes					
Position	1236		2677	3435	
Nucleotide change	C > T	G > A	G > T	C > T	
Amino acid change		A893T	A893S		
		a			
*1					
*2	T		T	T	
*4	T			T	
*6				T	
*8	T				
*9	T		T		
*10		A			
*11	T	A			
*18		A		T	

*Specific for Asian populations.

added novel haplotypes in other blocks after assessing a large number of subjects.

LD analysis revealed that one of the marker SNPs in the promoter region, -1789G>A, was moderately linked to IVS5 + 123A>G, previously classified into Block 2. Therefore, we shifted the border between Block 1 and Block 2 and re-analyzed the Block 1 haplotypes. Two promoter haplotype classes associated with functional changes have been reported previously (Taniguchi *et al.* 2003; Takane *et al.* 2004). One class included the -1789G>A SNP, and the other included the three linked SNPs of -1847T>C, -1347T>C and -129T>C. In our analysis these SNPs were included in our Block 1 region.

The haplotype containing -1789G>A was reported to be associated with reduced P-gp expression levels in the colon and liver, and reduced promoter activity was shown in a reporter gene assay (Taniguchi *et al.* 2003) (see Fig. 7). However, another study found that a haplotype containing -1789G>A without -145C>G (subgroups B and J in our present study) showed no change in the reporter assay, while another haplotype

that contained -1789G>A together with -145C>G (subgroup L) showed reduced promoter activity (Takane *et al.* 2004). Data on the functional effects of haplotypes harbouring the three linked SNPs (G and E subgroups) are also contradictory. While one study showed an association with reduced colon and liver P-gp expression levels in patients and reduced promoter activity in a reporter gene assay (Taniguchi *et al.* 2003), another study reported an association with increased P-gp expression levels in the placenta and liver, and with increased promoter activity in a reporter gene assay (Takane *et al.* 2004). By expanding Block 1 into intron 5 we identified additional types within previously reported wild-type sequences (corresponding to subgroup A in this study) and other variant haplotypes (subgroups B, E, G, J, and L) (Fig. 7). In total our data revealed 11 tagging variations in Block 1: -1789G>A, -1461_-1457CATCdel, -371A>G, -145C>G, -129T>C, IVS1 - 78delG, IVS4 - 25G>T, 304G>C (G102R), 325G>A (E109K), IVS + 76T>G and IVS5 + 123A>G. Thus, if some of these markers are of functional importance it is possible that

our subdivisions (types within A and other subgroups) might explain the discrepancies in P-gp expression levels in the previously reported studies. In fact, our preliminary observation has suggested possible influences of some of the tagging variations in Block 1 on pharmacokinetic parameters of paclitaxel (data not shown). However, this hypothesis requires further clarification in large scale clinical studies.

Several novel haplotypes were added to the other 3 blocks (1, 38, and 4 new haplotypes in Blocks -1, 2, and 3, respectively). We identified a new haplotype *1d in Block -1, but this variant haplotype was very rare and the functional significance of uncommon Block -1 haplotypes remains unknown. We added 7 new groups to Block 2 haplotypes (*12 to *18), but their frequencies were also very low (0.002 and less). We also confirmed the previous finding that, in order of frequency, the major groups were *2d, *10a, *1e, and *8a. In our previous study we estimated the relative P-gp activity of the different haplotypes according to the renal clearance of irinotecan and its metabolites in Japanese cancer patients. While we found a significant association between *2, which contained the three common SNPs, and reduced renal clearance levels, associations with the *6, *8, and *10 groups that contained only one of the common markers remained unclear. For the *4, *9, and *11 groups, which harbour two marker SNPs in Block 2, functional evaluation was impossible due to the small number of subjects. Previously we showed that *1f may have been associated with reduced P-gp activity. The current study revealed that *1f in Block 2 was completely linked with the newly defined *1d in Block 1, which contained IVS4 -25G>T. A further association study is needed to clarify the effects of the linked *1d (Block 1) and *1f (Block 2) haplotypes. Regarding Block 3 we added several minor *1 haplotypes and confirmed the previous findings that *1a and *1b were the major haplotypes. We previously observed a trend for an association between *1b and higher P-gp activity. Taking into consideration the haplotype-combinations across the blocks this trend also needs to be confirmed in a larger number of subjects.

It is well recognized that there are large ethnic differences in the frequencies of functionally important haplotypes, including 1236C>T, 2677G>T, and 3435>T (corresponding to the *2 group in Block 2), and pro-

moter region SNPs (corresponding to the variant Block 1 subgroups). Comparison of our data with the results from other ethnic groups indicated the existence of unique haplotype profiles in the Japanese population. As suggested by the previous report on the promoter region (Takane *et al.* 2004), Japanese samples exhibited large variations in Block 1 haplotypes. This suggested that not only *2 in Block 2 but also certain Block 1 haplotypes may be functionally important in the Japanese ethnic group. For Block 2 we confirmed our previous findings that the major groups were *1 and *2, and that *2d was the most frequent haplotype. While both groups were detected as the major types in other Asian and Caucasian populations, *1 was considerably more frequent than *2 in Africans (Kroetz *et al.* 2003). Another recent study found that the two major haplotypes were common to 5 ethnic groups (Tang *et al.* 2004). That study also revealed that the Chinese and Malay haplotype profiles were very similar, and that while some similarities were also observed between Caucasian and Indian populations, Africans differed from all other non-African populations. Furthermore, their study suggested that positive selection for 2677T-3435T had occurred in Chinese and Malays, and for 3435C in Africans. As pointed out previously, frequent occurrence of *10 (2677G>A) was unique to Japanese compared with Caucasians and African populations. Our study revealed higher frequencies of *10 (2677G>A) and *6 (3435C>T) and lower frequencies of *8 (1236C>T) and *9 (1236C>T and 2677G>T) than reported for Asian populations in Kroetz *et al.* (2003) (Table 5). This difference might be due to the mixed Asian population used in the report, as differences in the frequencies of 2677G>A between the Chinese, Malay, and Indian populations have been noted (Tang *et al.* 2004). The finding that the high frequency of 2677G>A is shared among Japanese, Koreans (Yi *et al.* 2004) and Chinese (Tang *et al.* 2004) suggests a close evolutionary relationship between these three populations.

A whole-genome haplotype database for three populations is now available at the Perlegen website (www.perlegen.com), which provides a good tool for investigation of the structures of human genetic variation within and between different populations (Hinds *et al.* 2005). For the *ABCB1* gene, however, we could not directly compare their data with ours because their