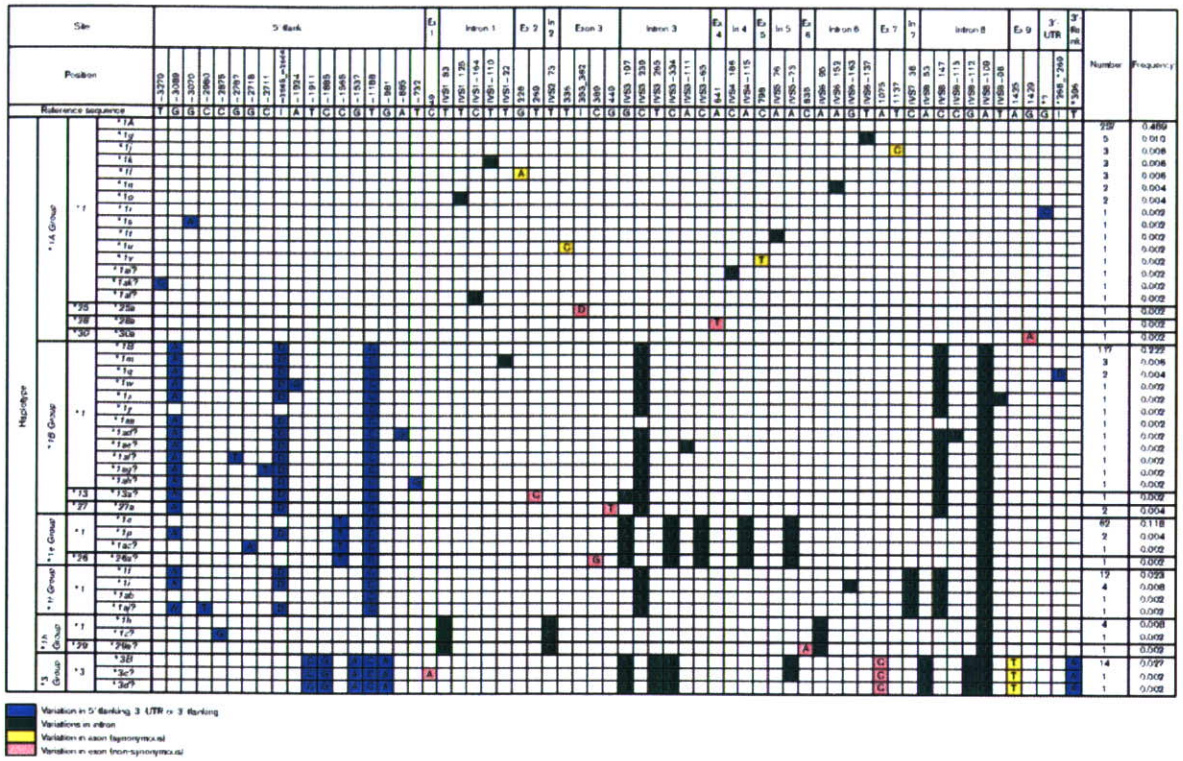
**Fig. 4**




Haplotype distribution of *CYP2C9* in a Japanese population. The positions on the cDNA sequence (A of the translational start codon is +1, based on NT_030059.12) or positions from the nearest exon were used for the description of the single nucleotide polymorphisms. The haplotypes already assigned by Human Cytochrome P450 Allele Nomenclature Committee are shown by capital alphabetical letters. The other inferred haplotypes are shown with lower case letters. The haplotypes inferred in only one patient are indicated with a question mark. White boxes denote the reference sequence; the colored squares represent intronic (green), non-coding (blue), synonymous (yellow) and non-synonymous (pink) sites. D, Deletion; I, insertion.

the eight SNPs, −1565C > T, −1188T > C, IVS3 + 197 G > A, IVS3−334C > T, IVS3−65G > C, IVS4−115A > G, IVS5−73A > G and IVS8−109A > T. The third common haplotype in Japanese was **1e* (0.118 in frequency).

The **1f* group, which was closely related to the **1B* group, included **1f*, **1i*, **1ab* and **1aj*. The **1f* harbors IVS7 + 38C > T together with all six SNPs in **1B*. The **1f* was inferred at a frequency of 0.023, while others were less than 0.01.

Although the frequency of the **1h* group was low ( < 0.01 in frequency), this group was quite different from any other group. All three haplotypes in this group, **1h*, **1z* and **29a* (Pro279Thr), contain three perfectly linked SNPs, IVS1 + 83T > C, IVS2 + 73T > C and IVS6 + 95A > G, together with the common SNP IVS8−109A > T. Except for **1h*, the other two haplotypes, **1z* and **29a*, were inferred in only one patient.

In the **3* group, three haplotypes harboring 1075A > C (Ile359Leu), **3B*, **3c* and **3d*, were inferred. King *et al.* [16] already assigned **3A* and **3B* in Caucasians; **3A* harbors the four SNPs in the promoter region, −1911T > C, −1885C > G, −1537G > A and −981G > A, together with 1075A > C (Ile359Leu), while **3B* harbors the additional SNP −1188T > C as well as the five SNPs described above. Because 1075A > C (Ile359Leu) was completely linked with −1188T > C, **3A* was not found in Japanese. The **3B* was found at a frequency of 0.027 and was the fourth frequent haplotype following **1A*, **1B* and **1e* and harbored IVS3 + 197G > A, IVS3 + 265T > C, IVS3−334C > T, IVS5−73A > G, IVS8 + 53A > T, IVS8−112G > A, IVS8−109A > T, 1425A > T (Gly475Gly) and *396T > A in addition to the five promoter SNPs and 1075A > C (Ile359Leu). The concurring SNPs in **3B* are in good agreement with the result of Veenstra *et al.* [13], who detected this haplotype in European-American patients and designated it Haplotype 3. Leu17Ile was assigned to haplotype **3c* concurring with 15 linked SNPs in **3B*. The **3c* and **3d* in this group were rare and ambiguous.

The network analysis of unambiguous haplotypes was performed to obtain a cladogram based on the sites and numbers of mutational events. In Fig. 5, the cladogram clearly discriminated six discrete haplotype groups, **1A*, **1B*, **1e*, **1f*, **1h* and **3*, which were connected to each other by several mutational sites. In the cladogram, each variation appears only once except for the two SNPs, −3089G > A and −2665_−2664delTG, which appeared twice, suggesting that recurrent recombination had hardly occurred throughout the analysed region. As a result, the haplotype structure of *CYP2C9* in Japanese is not complex, and only five common haplotypes were found in more than 10 subjects (at frequencies > 0.02), **1A*, **1B*, **1e*, **1f* and **3B*, account for 87% of all observed haplotypes. The haplotype-tagging SNPs (htSNPs) that resolved the five common haplotypes were the following four variations: −1565C > T, 1075A > C (Ile359Leu), IVS7 + 38C > T and IVS8 + 147C > T, which are indicated in red in Fig. 5.

Based on the haplotype structure in Caucasians reported by Veenstra *et al.* [13], we added **2* (430C > T, Arg144Cys) in the cladogram. The **2* was separated on the way from the long branch towards **3* and distinguished from **3* by multiple SNPs.

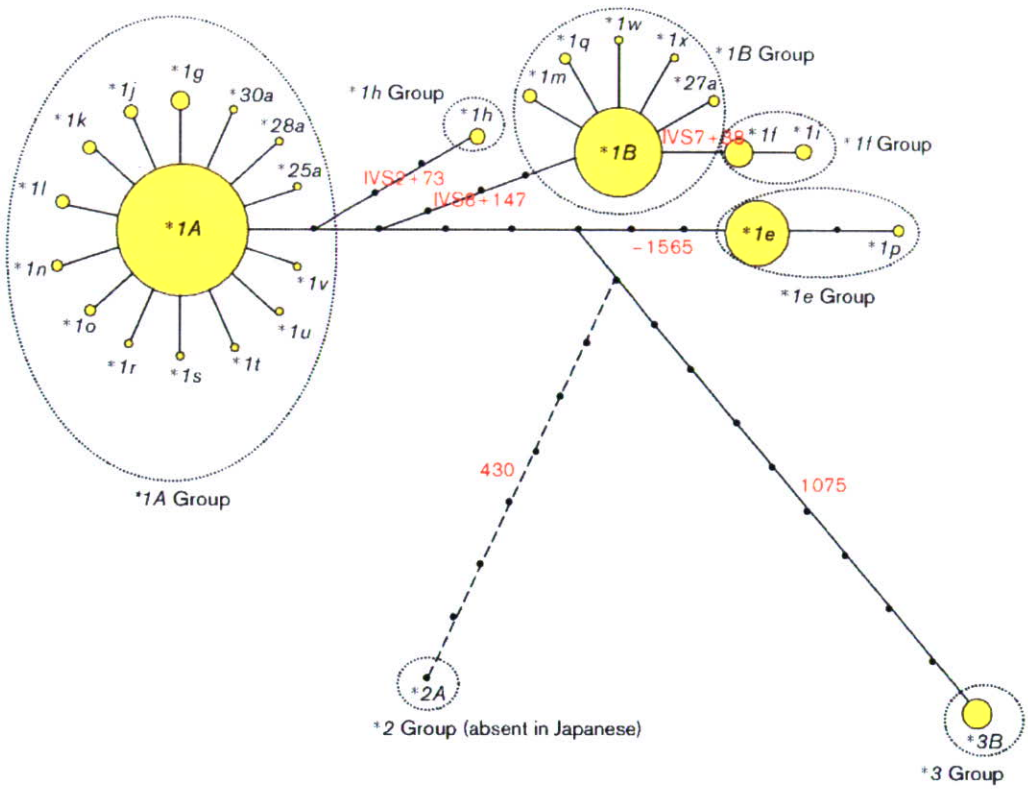
**Ethnic differences in haplotype distribution**

The haplotype distribution in Japanese was compared with those in other ethnic groups reported previously [10,13]. As shown in Table 4, frequencies of several *CYP2C9* haplotypes in Japanese were comparable to those in Asians, but quite different from those of corresponding haplotypes found in Caucasians and Africans. The frequency of the wild-type haplotype, **1A*, was higher in Japanese (0.489) than that in Caucasians (0.281) reported by Veenstra *et al.* [13]. The haplotype **1B*, which was tagged by IVS8 + 147C > T, was inferred at a frequency of 0.222 in Japanese. This frequency is comparable to that in Caucasians (0.175). The third dominant haplotype in Japanese, **1e* tagged by −1565C > T, was not found in Caucasians. According to Blaisdell *et al.* [10], the haplotypes harboring −1565C > T, were inferred with a frequency of 0.043 in Asians (Haplotype P) and with a frequency of 0.133 in African-Americans (Haplotype S), but not in Caucasians. Therefore, the absence of the haplotype **1e* appears to be characteristic of Caucasians. The haplotype **1f*, tagged by IVS7 + 38C > T, was not found in Caucasians. Blaisdell *et al.* [10] inferred Haplotype O harboring IVS7 + 38C > T with a frequency of 0.022 in Asians, which was comparable to that in Japanese (0.023). Therefore, the distribution of **1f* might be restricted in Asians.

A marked difference in haplotype frequencies of **1h* was observed between Japanese and Caucasians. Its frequency was approximately 20-fold higher in Caucasians than in Japanese. Blaisdell *et al.* [10] reported that the frequencies of Haplotype E harboring IVS2 + 73T > C was 0.043, 0.100 and 0.205 in Asians, African-Americans and Caucasians, respectively. Therefore, the haplotype **1h* appears to be more frequent in Caucasians and African-Americans than in Asians.

The frequency of haplotype **3B* harboring Ile359Leu in Japanese (0.027) was comparable to that in Asians (0.022; Haplotype U) reported by Blaisdell *et al.* [10], but was slightly lower than those in Caucasians reported by Veenstra *et al.* [13] (0.057; Haplotype 3), by King *et al.*

**Fig. 5**




Network analysis of unambiguous haplotypes in *CYP2C9*. The areas in the circles represent the approximate frequencies of each haplotype. The htSNPs that discriminate the common haplotypes are indicated in red. The **2A* haplotype found only in Caucasians was connected in this cladogram based on the report by Veenstra *et al.* [13].

**Table 4 Ethnic differences in *CYP2C9* haplotypes**

| Haplotype tagging single nucleotide polymorphisms | Present study | | Veenstra *et al.* [13] | | Blaisdell *et al.* [10] | | | |
|---|---|---|---|---|---|---|---|---|
| | Haplotype | 263 Japanese subjects | Haplotype | 192 European-American patients | Haplotype | 23 Asians | 15 African-Americans | 22 Caucasians |
| None | **1A* | 0.489 | 7 (**1A*) | 0.281 | V (=**1A*) | 0.522 | 0.333 | 0.386 |
| IVS8+147C>T | **1B* | 0.222 | 6 (**1B*) | 0.175 | J | 0.304 | 0.067 | 0.091 |
| -1565C>T | **1e* | 0.118 | - | - | S, P | 0.043 | 0.133 | ND |
| IVS7+38C>T | **1f* | 0.023 | - | - | O | 0.022 | ND | ND |
| IVS2+73T>C | **1h* | 0.008 | 14, 15, 16, 17, 22 | 0.211 | E | 0.043 | 0.100 | 0.205 |
| 430C>T (Arg144Cys) | **2* | ND | 20 (**2A*) | 0.107 | T (=**2*) | ND | ND | 0.068 |
| 1075A>C (Ile359Leu) | **3B* | 0.027 | 3 | 0.057 | U (=**3B*) | 0.022 | ND | 0.159 |
| IVS6-32T>C | - | ND | 8 | 0.063 | G | ND | ND | 0.068 |

ND, Not detected.

[16] (0.062; Haplotype 5, **3B*), or by Morin *et al.* [18] (0.081). On the other hand, the distribution of the haplotype harboring IVS6–32T > C appears to be restricted in Caucasians. Its frequency in Caucasians was 0.063 (Haplotype 8) by Veenstra *et al.* [13] and 0.068 (Haplotype G) by Blaisdell *et al.* [10]. This result was consistent with the HapMap data where IVS6–32T > C was found in Europeans with an allele frequency of 0.067, but not in Yoruba, Han Chinese and Japanese.

### Relationship between *CYP2C9* and *CYP2C19* haplotypes

The CYP2C subfamily members (*CYP2C18*, *CYP2C19*, *CYP2C9* and *CYP2C8*) are located on chromosome 10q24 as a cluster that spans approximately 400 kb. Previously, we reported the genetic variations and haplotype structure of *CYP2C19* in a Japanese population [29]. Because 253 out of the 263 subjects in this study were identical to those in the previous study on *CYP2C19*, we analysed LD patterns and the associations of haplotypes between *CYP2C9* and *CYP2C19*. Of all the 1225 pairwise $|D'|$ values between 50 common SNPs consisting of 24 in *CYP2C19* [29] and 26 in *CYP2C9* ( > 0.01 in their allele frequencies), 988 pairs (80%) had $|D'| > 0.90$ (data not shown), indicating an extended LD block covering both *CYP2C19* and *CYP2C9*. As shown in Table 5, 92% (228/249) of the wild-type haplotype **1A* was linked with *CYP2C19*1d*, which was the most dominant *CYP2C19* haplotype in Japanese harboring 99C > T (Pro33Pro) and 991A > G (Ile331Val). The majority of **1B* (83%, 94/113) was linked with *CYP2C19*2c*, which was the second dominant *CYP2C19* haplotype in Japanese harboring 681G > A (a splicing defect). The majority of **1e* (92%, 54/59) was linked with *CYP2C19*3b*, which was the third dominant *CYP2C19* haplotype in Japanese harboring 636G > A (Trp212X). The majority of **3B* (92%, 12/13) was linked with *CYP2C19*1e* without any non-synonymous amino acid change (Ile at codon 331). Namely, intergene haplotypes (*CYP2C19–CYP2C9* combinations) were found at the following frequencies (Table 6): *CYP2C19*1d–CYP2C9*1A* (0.451), *CYP2C19*2c–CYP2C9*1B* (0.186), *CYP2C19*3b–CYP2C9*1e* (0.107), *CYP2C19*1e–CYP2C9*3B* (0.024) and *CYP2C19*2c–CYP2C9*1f* (0.02). A strong linkage between *CYP2C19* and *CYP2C9* haplotypes indicated rare recombination between these two genes.

## Discussion

The present study provides comprehensive data on genetic variations of *CYP2C9*, which encodes a clinically important enzyme that metabolizes numerous therapeutic drugs with a narrow therapeutic index. We found 62 variations, including seven novel non-synonymous ones, in 263 Japanese subjects. To assess the effects of these novel variations on both protein expression levels and enzymatic activity, we transiently expressed the recombinant protein in COS-1 cells. Although the expression levels of recombinant proteins in a mammalian expression system were low as compared with bacterial and baculovirus-mediated systems, the mammalian system is reliable in assessing functional significance of the CYP variants because of the correct protein folding assured by mammalian chaperone proteins. Indeed, the observed $K_m$ value in our system was comparable to that in human liver microsomes when diclofenac was used as a probe drug [22,27].

The **25* (Lys118ArgfsX9), which was found in a heterozygous diabetic patient treated with glimepiride, produced an early termination codon within the C-helix. The variant protein for Lys118ArgfsX9 was not detected by Western blot analysis, suggesting that it was a null allele. Two null alleles with nonsense mutations in *CYP2C9* were already reported: **6* (Lys273ArgfsX34) [9] and **15* (Ser162X) [12]. An African-American woman with homozygous **6* showed severe phenytoin toxicity. On the other hand, an Indian woman with heterozygous **15* required a typical warfarin maintenance dose. We are now evaluating the clinical effects of Lys118ArgfsX9 on pharmacokinetic/pharmacodynamics profiles of glimepiride.

**Table 5 Frequencies of common haplotype combinations of *CYP2C19* and *CYP2C9***

| Haplotype Number (frequency) | *2C9*1A* 249 (0.492) | *2C9*1B* 113 (0.223) | *2C9*1e* 59 (0.117) | *2C9*3B* 13 (0.026) | *2C9*1f* 12 (0.024) |
|---|---|---|---|---|---|
| *2C19*1d* 249 (0.492) | **228 (0.451)** | – | – | – | – |
| *2C19*2c* 122 (0.241) | – | **94 (0.186)** | – | – | **10 (0.020)** |
| *2C19*3b* 58 (0.115) | – | 2 (0.004) | **54 (0.107)** | – | – |
| *2C19*1e* 22 (0.043) | – | 8 (0.016) | – | **12 (0.024)** | – |
| *2C19*1f* 11 (0.022) | 7 (0.014) | – | – | – | – |
| *2C19*1g* 7 (0.014) | 7 (0.014) | – | – | – | – |

Number and frequencies (within parenthesis) of various haplotype combinations of *CYP2C19* and *CYP2C9* are shown as a matrix with *CYP2C19* haplotypes as rows and *CYP2C9* haplotypes as columns. Frequencies representing major combinations are shown in bold. *CYP2C19* haplotypes in a Japanese population are defined by Fukushima-Uesaka *et al.* [29].

The **26* (Thr130Arg) exhibited a drastic decrease in $V_{max}$ against diclofenac hydroxylation without a significant change in the $K_m$ value. Thr130 is not within the substrate recognition sites (SRSs), but is highly conserved in the CYP2C family [30]. The crystal structure of CYP2C9 showed that Thr130 in the C-helix is on the surface of the protein [31,32], suggesting little or no importance for this residue in substrate binding. Around Thr130Arg, two SNPs, **2* (Arg144Cys) in the D-helix and **14* (Arg125His) in the C-helix, were already reported, both of which are located on the exterior of the protein. The **2* causes a small decrement in $V_{max}$ (0–35%) and little or no change in the $K_m$ for catalysis of various substrates [5]. An *in vitro* experiment has suggested that the observed reductions in $V_{max}$ of **2* might be associated with the altered interaction of the recombinant protein with cytochrome P450 reductase [33]. According to Delozier *et al.* [34] recombinant **14* exhibited a five-fold increase in the $K_m$ value and a 65% decrease in the $V_{max}$ towards tolbutamide. They suggested that loss of activity might reflect altered affinity of recombinant protein for the coenzyme because the previous site directed mutagenesis study demonstrated that the corresponding residue of CYP2B4 in the C-helix plays a prominent role in binding its redox partners, cytochrome b5 and P450 reductase [35]. Thus, we speculate that the substitution of Thr130 with the positively charged arginine might influence an electrostatic interaction between CYP2C9 and P450 reductase and/or cytochrome b5 as proposed in Arg144Cys [33] and Arg125His [34].

The **28* (Gln214Leu), which was found in a heterozygous diabetic patient, exhibited an approximately two-fold increase in the $K_m$ value and a more than 50% decrease in the $V_{max}$, resulting in a 77% decrease in intrinsic clearance. Gln214, which is conserved in the CYP2C family, is located between the F- and G-helix and only five amino acids downstream of SRS 2 [30]. No site directed mutagenesis experiments were performed to examine the functional importance of the F-G loop of CYP2C9. Furthermore, there was significant inconsistency in the conformation in the F-G loop region between two crystal structures available for human CYP2C9, 1OG5 with warfarin [31] and 1R9O with flurbiprofen [32]. In the 1OG5 structure, in which seven mutations were introduced for crystallization, two short helices F′ and G′ were resolved, and Gln214 was located in the additional F′ helix. By contrast, helix F′ and helix G′ were not evident in the 1R9O structures, which exhibited a more extended conformation of the region between the F-G loop and helix A although seven amino acids (Gly214-Ser220) of the F-G loop were not included in the model. The structural difference in the F-G loop between the 1R9O and 1OG5 are likely to reflect the conformational flexibility indicative of an adaptive fit to the various substrates with different sizes, polarity and stereochemical features. In this regard, this is the first report experimentally demonstrating that Gln214 in the F-G loop affects the metabolism of diclofenac.

The **30* (Ala477Thr) was found in a heterozygous healthy volunteer. This substitution showed a similar extent of defective catalytic activity for **28* (Gln214Leu) with regard to the $K_m$, $V_{max}$ and intrinsic clearance. Ala477 is within the substrate recognition site 6 (SRS 6) and forms a β4–2 sheet [30]. The importance of Phe476, which is next to Ala477, was previously proposed on the basis of a combined protein and pharmacophore model for CYP2C9 [36]. Supporting this model, Melet *et al.* [37] showed by site-directed mutagenesis that the Phe476Ile variant was two times less efficient than the wild-type CYP2C9 in terms of diclofenac 4′-hydroxylation without affecting the protein expression levels, which resulted from 3.7- and 2-fold increases in the $K_m$ and $K_{cat}$ values, respectively. Furthermore, the Phe476Ile variant exhibited a significant change in the regioselectivity of diclofenac hydroxylation; namely, this substrate was also 5-hydroxylated [37]. They concluded that Phe476 played a crucial role in substrate recognition and hydroxylation of diclofenac by CYP2C9, presumably via π-stacking interactions between its phenyl residues and substrate aromatic rings. Our results indicated that Ala477 located next to Phe476 might also be important for substrate recognition and hydroxylation of diclofenac. The substitution of small alanine to the nucleophilic residue, threonine, might influence the hydrophobic interaction of Phe476 with the substrates. Although we did not investigate the effects of Ala477Thr on the regioselectivity of diclofenac hydroxylation because of no availability of 3′- and 5′-hydroxy metabolites, further studies would be needed to provide detailed features of the CYP2C9 active site.

The other three novel alleles, Leu17Ile, **27* (Arg150Leu) and **29* (Pro279Thr), showed similar catalytic activities towards diclofenac hydroxylation as the wild-type. Because recombinant CYP2C proteins without their N-terminus have been found to be catalytically active, it is reasonable that a conservative substitution, Leu17Ile, in the membrane anchor region had no significant effects on both microsomal expression level and catalytic activity. Blaisdell *et al.* [10] also reported that an adjacent substitution, **7* (Leu19Ile) had no effect on catalytic activity towards tolbutamide *in vitro*. However, it should be noted that the diabetic patient carrying Leu17Ile was heterozygous for Ile359Leu, and both alleles were assigned to the same haplotype (**3c*) by an expectation-maximization algorithm. Because Ile359Leu itself is functionally definitive [5], the combined effects of Leu17Ile and Ile359Leu on clinical phenotype should be carefully estimated *in vivo*.

On the other hand, no apparent effects on the catalytic activity of **27* (Arg150Leu) were a little surprising because a substitution in the same position, **8* (Arg150His), exhibited a modest decrease in $K_m$ and a two-fold increase in clearance of tolbutamide [10]. Because Arg150 is a surface residue of the D-helix, different electrostatic status near the substituted residues (His verses Leu) might differently influence the substrate-dependent catalytic behaviour. Pro279, located between helices H and I, is not conserved in the CYP2C family and is unlikely to play an important role in catalytic activity of CYP2C9. Accordingly, it was reasonable that **29* (Pro279Thr) did not show any functional changes.

The two previously reported alleles, **3* (Ile359Leu) and **13* (Leu90Pro), were also found in Japanese at allele frequencies of 0.03 and 0.002, respectively. Experiments performed *in vivo* and *in vitro* consistently demonstrated that **3* was associated with substantial loss of enzyme activity that resulted from decreased $V_{max}$ and increased $K_m$ for many CYP2C9 substrates [5]. The **13*, first detected in a Chinese poor metabolizer of lornoxicam, has been found at an allele frequency of 0.01 in a Chinese population [11]. Guo *et al.* [38] revealed that the Leu90Pro substitution markedly decrease the intrinsic clearance of lornoxicam *in vitro* and *in vivo*. Except for **3* and **13*, the other 21 *CYP2C9* alleles published on the Human CYP Allele Nomenclature Committee homepage were not found in the present study. Taken together, approximately 8% of Japanese individuals (21 out of the 263 subjects) carry one of the functionally defective alleles: **3* (Ile359Leu), **13* (Leu90Pro), **25* (Lys118ArgfsX9), **26* (Thr130Arg), **28* (Gln214Leu) and **30* (Ala477Thr). The existence of several rare but defective alleles in South-east Asian subjects was also confirmed by DeLozier *et al.* [34], such as **15* (Ser162X), **18* (Asp397Ala), **14* (Arg125His) and **16* (Thr299Ala). Therefore, defective alleles of *CYP2C9*, including **3*, occur more frequently than previously expected in Japanese and probably Asians. Thus, this could in part account for the interethnic and/or interindividual variability in metabolizing CYP2C9 substrate drugs.

Recently, Veenstra *et al.* [13] reported the first whole-gene high-resolution haplotype structures of *CYP2C9* in European-American patients administrated warfarin. They determined 23 haplotypes, only eight of which occurred at a frequency greater than 5%, indicating the overall haplotype structure of *CYP2C9* was not complex. Apart from distinctive ethnic differences in haplotype frequencies, the results of the present study are consistent with this report. The overall haplotype structure of *CYP2C9* in Japanese was also simple: only five common haplotypes with a frequency $>2\%$ accounted for most of the haplotypes, and they can be distinguished by only four htSNPs.

As shown in Table 5, each of the five common *CYP2C9* haplotypes in Japanese (**1A*, **1B*, **1e*, **1f* and **3B*) was strongly linked with each of the four major *CYP2C19* haplotypes (**1d*, **2c*, **3b* and **1e*) reported by Fukushima-Uesaka *et al.* [29]. This result is in good agreement with a recent report by Ahmadi *et al.* [20] and Walton *et al.* [39], in which the long-range LD spanning *CYP2C19* and *CYP2C9* was identified. The *CYP2C19–CYP2C9* haplotype combination, *CYP2C19*1d–CYP2C9*1A* (0.451 in frequency), is prevalent in Japanese. It is the combination of the most dominant haplotypes of both *CYP2C19* and *CYP2C9* in Japanese that are associated with extensive metabolic phenotypes. There is no linkage between *CYP2C9*3B* and the two defective haplotypes of *CYP2C19*, *CYP2C19*2c* and *CYP2C19*3b*, suggesting that Japanese individuals do not have a haplotype simultaneously resulting in poor metabolism phenotypes for both CYP2C19 and CYP2C9. However, approximately 67% of Japanese individuals bear one or two copies of haplotypes harboring either *CYP2C19*2* (681G > A, splicing defect), *CYP2C19*3* (636G > A, Trp212X), or *CYP2C9*3* (1075A > C, Ile359Leu). The very close associations between *CYP2C19* and *CYP2C9* haplotypes could complicate pharmacogenetic studies on drugs such as phenytoin, tolbutamide and chlorpropamide that are metabolized by both CYP2C9 and CYP2C19.

In summary, we identified 62 variations, including 32 novel ones, in *CYP2C9* from Japanese subjects. Seven novel SNPs with non-synonymous substitutions were found, of which one, *CYP2C9*25* (Lys118ArgfsX9), was a null allele, and three, *CYP2C9*26* (Thr130Arg), *CYP2C9*28* (Gln214Leu) and *CYP2C9*30* (Ala477Thr), were functionally defective alleles towards diclofenac. Further clinical studies will be required to determine the clinical importance of the novel CYP2C9 alleles, including promoter variations, for the metabolism of CYP2C9 substrate drugs. Furthermore, remarkable differences in haplotype distributions among ethnic groups have highlighted the importance of ethnic-specific pharmacogenetic data.

## Acknowledgements

We thank Drs Yasushi Kaburagi and Sachiko Honjo at the hospital, International Medical Center of Japan, for acquiring informed consent from the patients. We also thank Ms Chie Sudo for her secretarial assistance.

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doi: 10.1111/j.1469-1809.2006.00260.x

# 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

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## 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

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 (Siegsmund *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 studies 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 (Johne *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[a] | | | |
| 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 | AAACTTCAGACGTCAGATCA |
| MDR1-P4F | GAAACATCCTCAGACTATGC | MDR1-P4R | CAGGAGGAATGTTCTGGCTT |
| Sequencing | | | |
| MDR1-P5F | ATTTCTTTGAAGTGCTTGGC | MDR1-P5R | GCCACCACCACTTCTGTCAA |
| MDR1-P6F | GATCTTTACCTGATGCTCAA | MDR1-P6R | GTGCCTATGATCTCTGTTTT |
| 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).
[a]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 $\beta$-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), except 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). Diplotype configurations (combinations of haplotypes) in each block were inferred by LDSUPPORT software, which determined the posterior probability distribution of diplotype 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 nonsynonymous 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'−1457del CATCC, 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 | | Site | Position | | Nucleotide change | Amino acid change | Frequency |
|---|---|---|---|---|---|---|---|---|
| | This study[a] | Reference | | NT_007933.14 | cDNA-based | | | |
| Block −1 | MJ[6.AB1078 | (novel) | 5′-Flanking | 12472468..12472461 | −8128..−8121 | GTAAAGTCAGATCTAACCAA/-CTGTTCATTGGT | | 0.002 |
| | MJ[6.AB1079 | (novel) | 5′-Flanking | 12466729 | −2389 | CTCCCATAGATAC/TATATAGAACAGA | | 0.001 |
| | MJ[6.AB1080 | b) | 5′-Flanking | 12466680 | −2340 | ATGTTGTCAGAGT/CATAGACAAGTTG | | 0.001 |
| | MJ[6.AB1081 | (novel) | 5′-Flanking | 12466659 | −2319 | GTTGGTGAAATGG/TCTACATGAGAGC | | 0.001 |
| | MJ[6.AB1072 | b,c) | 5′-Flanking | 12466187 | −1847 | GTTTAGCGAGGGT/CTTAAGGCCATTC | | 0.084 |
| | MJ[6.AB1073 | rs12720464 [d] | 5′-Flanking | 12466129 | −1789 | AATGAAAGGTGAG/AATAAAGCAACAA | | 0.204 |
| | MJ[6.AB1082 | (novel) | 5′-Flanking | 12466065 | −1725 | AAGATTAAAAACG/ACATGTAATGAAG | | 0.001 |
| | MJ[6.AB1083 | (novel) | 5′-Flanking | 12465983 | −1643 | CAGTGAACAATGC/TTGTACACTTGCA | | 0.001 |
| | MJ[6.AB1084 | (novel) | 5′-Flanking | 12465806 | −1466 | GGTCAGGAGATCA/GAGACCATCCTGG | | 0.002 |
| | MJ[6.AB1085 | c) | 5′-Flanking | 12465801..12465797 | −1461..−1457 | GGAGATCAAGACCATCC /-TGGCTAACACAG | | 0.030 |
| Block 1 | MJ[6.AB1074 | b,c) | 5′-Flanking | 12465687 | −1347 | GCAGGAGAATGGT/CGTGAACCCGGGA | | 0.084 |
| | MJ[6.AB1086 | (novel) | 5′-Flanking | 12465619 | −1279 | CCTCGCGCGACAAA/GGCAAGACTCCGT | | 0.004 |
| | MJ[6.AB1075 | b,c) | 5′-Flanking | 12465494 | −1154 | AGAAAAAATTAT/CGCCTTTGAAGTA | | 0.001 |
| | MJ[6.AB1087 | (novel) | 5′-Flanking | 12465444 | −1104 | ATCCTCAGACTAT/CGCAGTAAAAAAC | | 0.001 |
| | MJ[6.AB1088 | (novel) | 5′-Flanking | 12465421 | −1081 | ACAAAGTGATTTT/CCTTCTTCTAAAC | | 0.002 |
| | MJ[6.AB1089 | (novel) | 5′-Flanking | 12465405 | −1065 | CTTCTAAACTTAT/CGCAATAAACTGA | | 0.001 |
| | MJ[6.AB1090 | (novel) | 5′-Flanking | 12465326 | −986 | TCCTCTATGTTCA/GTAAGAAGTAAGA | | 0.001 |
| | MJ[6.AB1091 | (novel) | 5′-Flanking | 12464967 | −627 | TTATCATCAAATA/GAAGCATGAACAG | | 0.002 |
| | MJ[6.AB1092 | (novel) | Exon 2 | 12463728 | 49 | AAGAAGAACTTTT/CTTAAACTGAACA | F17L | 0.001 |
| | MJ[6.AB1093 | (novel) | Exon 4 | 12449246 | 144 | TTGGCTTGACAAG/TTTGTATATGGTG | K48N | 0.001 |
| | MJ[6.AB1094 | (novel) | Exon 5 | 12433798 | 304 | ATCAATGATACAG/CGGTTCTTCATGA | G102R | 0.005 |
| | MJ[6.AB1095 | (novel) | Exon 6 | 12430553 | 354 | TGCCTATTATTAC/TAGTGGAATTGGT | Y118Y | 0.001 |
| | MJ[6.AB1096 | (novel) | Exon 6 | 12430460 | 447 | CAAAATTAGAAAA/GCAGTTTTTTTCAT | K149K | 0.002 |
| | MJ[6.AB1097 | (novel) | Exon 12 | 12413771 | 1342 | TATGACCCCACAG/AAGGGGATGGTGA | E448K | 0.001 |

**Table 2** Continued.

| Block | SNP ID | | Site | Position | | Nucleotide change | Amino acid change | Frequency |
|---|---|---|---|---|---|---|---|---|
| | This study[a] | Reference | | NT_007933.14 | cDNA-based | | | |
| | MPJ6_AB1052 | e) | Intron 12 | 12413746 | IVS12 +17 | GATGACCCATGC**G/A**AGCTAGACCCTG | | 0.006 |
| | MPJ6_AB1098 | (novel) | Intron 12 | 12413720 | IVS12 +43 | GGTGATCAGCAG**T/G**CACATTGCACAT | | 0.001 |
| | MPJ6_AB1099 | (novel) | Intron 13 | 12413353 | IVS13 +90 | CTACTATAAATC**G/A**GAAGAAGGGAAA | | 0.001 |
| | MPJ6_AB1100 | (novel) | Exon 15 | 12409538 | 1804 | ATCGCTGGTTTC**G/A**ATGATGGAGTCA | D602N | 0.002 |
| | MPJ6_AB1101 | (novel) | Intron 15 | 12408686 | IVS15 −95 | GTTACTAAACAA**A/G**TTGCTGTTTTCC | | 0.001 |
| | MPJ6_AB1065 | (novel) | Intron 16 | 12408363 | IVS16 +52 | CTGTGGTTCCCT**A/C**GTTTGGTGGGCT | | 0.003 |
| | MPJ6_AB1102 | (novel) | Intron 16 | 12407939 | IVS16 −72 | TCCTTTACTAAT**T/A**TTTGTGCGTATG | | 0.001 |
| | MPJ6_AB1103 | (novel) | Intron 18 | 12404862 | IVS18 +87 | AGTGTAATTGGC**C/T**TTTTAGTAGAAC | | 0.001 |
| Block 2 | MPJ6_AB1104 | (novel) | Exon 19 | 12402898 | 2359 | ATCCTCACCAAG**C/T**GGCTCCGATACA | R787W | 0.001 |
| | MPJ6_AB1105 | (novel) | Intron 19 | 12400221 | IVS19 −88 | GGGGTATAAGTA**T/C**AAACAAAACTGA | | 0.001 |
| | MPJ6_AB1106 | (novel) | Intron 20 | 12395242 | IVS20 −153 | TTCTTACTGTAG**A/G**AACTCAATAAAC | | 0.001 |
| | MPJ6_AB1107 | (novel) | Intron 20 | 12395172 | IVS20 −83 | GAATATAGTCTC**A/G**TGAAGGTGAGTT | | 0.001 |
| | MPJ6_AB1108 | (novel) | Intron 21 | 12384544_12384541 | IVS21−73_−76 | TTATTTTCATTA**GTCT/-**GTTTTATAGAAT | | 0.003 |
| | MPJ6_AB1067 | (novel) | Exon 22 | 12384435 | 2719 | AACTTCCGAACC**G/A**TTGTTTCTTTGA | V907I | 0.002 |
| | MPJ6_AB1109 | (novel) | Intron 22 | 12384359 | IVS22 +9 | ACAGGTAATAAC**C/T**GCTGAAGAGTGG | | 0.001 |
| | MPJ6_AB1076 | f) | Exon 24 | 12380229 | 2956 | GTCTTTGGTGCC**A/G**TGGCCGTGGGGC | M986V | 0.001 |
| | MPJ6_AB1110 | (novel) | Exon 24 | 12380142 | 3043 | ATCATTGAAAAA**A/G**CCCCTTTGATTG | T1015A | 0.001 |
| | MPJ6_AB1111 | (novel) | Intron 26 | 12372831_12372834 | IVS26 +33_36 | ACAGCCTGGGAG**-/CATG**TGGCAGCCTCTC | | 0.001 |
| Block 3 | MPJ6_AB1112 | (novel) | Intron 26 | 12369713 | IVS26 −78 | ATATAGAATCGT**C/G**TATCCTACTTTC | | 0.001 |
| | MPJ6_AB1077 | rs2235051[d] | Exon 28 | 12367931 | 3747 | GTTTCAGAATGG**C/G**AGAGTCAAGGAG | 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.

[a]SNP ID assigned by our project team (MPJ-6).

[b]Taniguchi *et al.* 2003.

[c]Takane *et al.* 2004.

[d]NCBI dbSNP

[e]Itoda *et al.* 2002.

[f]Tanabe *et al.* 2001.

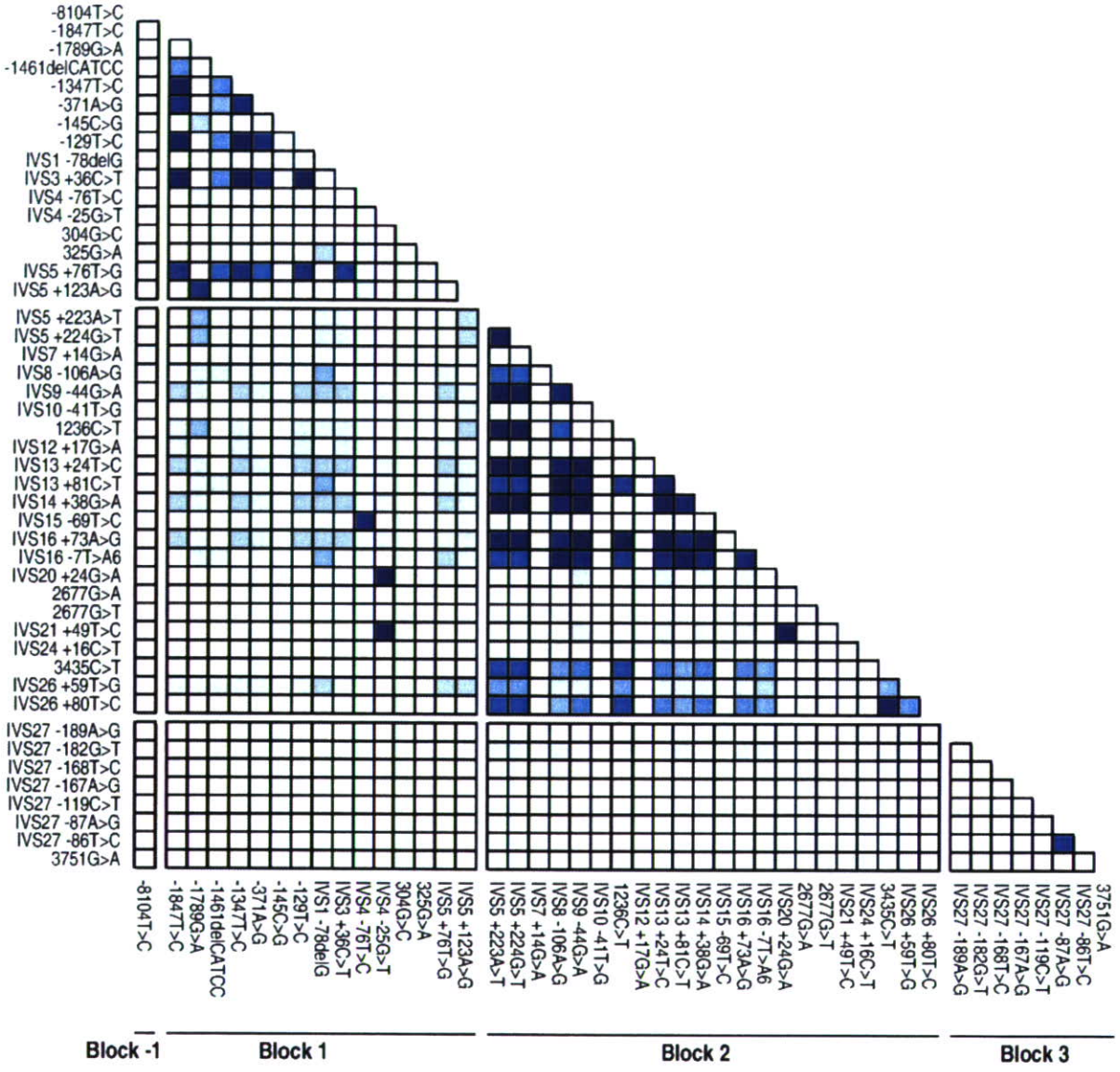



**Figure 1** Linkage disequilibrium (LD) analysis of the *ABCB1* gene. Pairwise LD ($r^2$ values) of the polymorphisms detected in 5 or more chromosomes is shown as a 10-graded blue colour.

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.

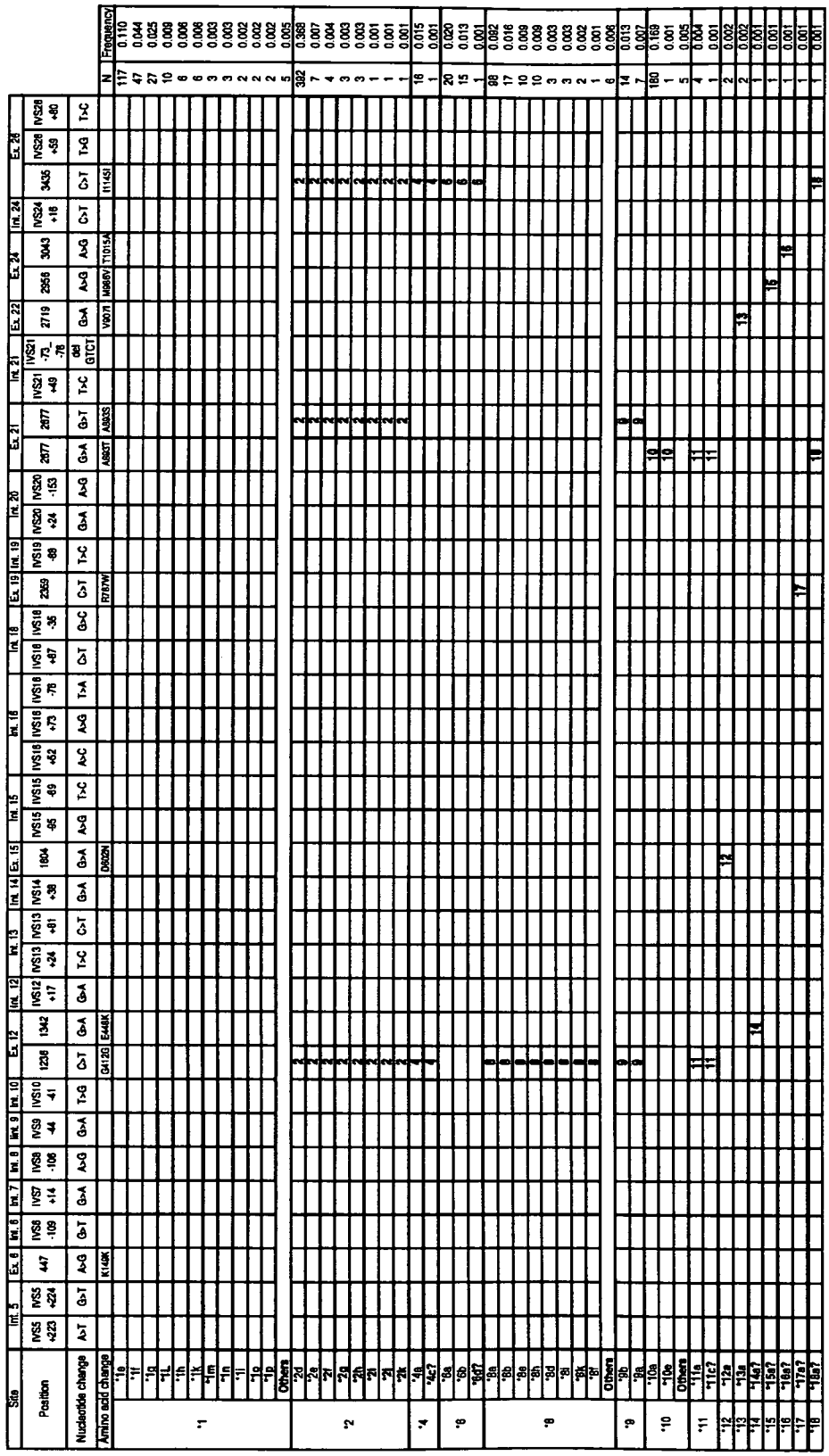
| Site | | 5'-Flanking | | | | |
|---|---|---|---|---|---|---|
| Position | | -8128 _8121 | -8104 | -7970 | | |
| Nucleotide change | | del CTAA CCAA | T>C | C>T | | |
| Amino acid change | | | | | N | Frequency |
| *1 | *1a | | | | 1053 | 0.988 |
| | *1b | | | | 2 | 0.002 |
| | *1c | | | | 9 | 0.008 |
| | *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 | | | | | | | | | | | | | | | Ex. 1(5'-UTR) | | Int. 1 | Ex. 2 | Int. 3 | Ex. 4 | Int. 4 | | Ex. 5 | | Int. 5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Position | | -2340 | -2319 | -1847 | -1789 | -1468 | -1481 _-1457 | -1347 | -1279 | -1081 | -1065 | -988 | -371 | -145 | -129 | IVS1 -78 | 49 | IVS3 +38 | 144 | IVS4 -76 | IVS4 -25 | 304 | 325 | IVS5 +78 | 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 |
| *1 | *1a | | | | | | | | | | | | | | | | | | | | | | | | | | 0.541 |
| | *1b | | | | | | | | | | | | | | | | | | | | | | | | | | 0.098 |
| | *1c | | | | | | | | | | | | | | | | | | | | | | | | | | 0.079 |
| | *1d | | | | | | | | | | | | | | | | | | | | | | | | | | 0.041 |
| | *1e | | | | | | | | | | | | | | | | | | | | | | | | | | 0.029 |
| | *1f | | | | | | | | | | | | | | | | | | | | | | | | | | 0.026 |
| | *1g | | | | | | | | | | | | | | | | | | | | | | | | | | 0.026 |
| | *1h | | | | | | | | | | | | | | | | | | | | | | | | | | 0.022 |
| | *1i | | | | | | | | | | | | | | | | | | | | | | | | | | 0.017 |
| | *1j | | | | | | | | | | | | | | | | | | | | | | | | | | 0.016 |
| | *1k | | | | | | | | | | | | | | | | | | | | | | | | | | 0.015 |
| | *1L | | | | | | | | | | | | | | | | | | | | | | | | | | 0.012 |
| | *1m | | | | | | | | | | | | | | | | | | | | | | | | | | 0.010 |
| | *1n | | | | | | | | | | | | | | | | | | | | | | | | | | 0.010 |
| | *1o | | | | | | | | | | | | | | | | | | | | | | | | | | 0.004 |
| | *1p | | | | | | | | | | | | | | | | | | | | | | | | | | 0.003 |
| | *1q | | | | | | | | | | | | | | | | | | | | | | | | | | 0.002 |
| | *1r | | | | | | | | | | | | | | | | | | | | | | | | | | 0.002 |
| | *1s | | | | | | | | | | | | | | | | | | | | | | | | | | 0.002 |
| | *1t | | | | | | | | | | | | | | | | | | | | | | | | | | 0.002 |
| | *1u | | | | | | | | | | | | | | | | | | | | | | | | | | 0.002 |
| | *1v | | | | | | | | | | | | | | | | | | | | | | | | | | 0.001 |
| | *1w | | | | | | | | | | | | | | | | | | | | | | | | | | 0.001 |
| | *1x | | | | | | | | | | | | | | | | | | | | | | | | | | 0.001 |
| | *1y | | | | | | | | | | | | | | | | | | | | | | | | | | 0.001 |
| | Others | | | | | | | | | | | | | | | | | | | | | | | | | | 0.009 |
| *2 | *2a | | | | | | | | | | | | | | | | | | | | | | 2 | | | | 0.017 |
| *3 | *3a | | | | | | | | | | | | | | | | | | | | | 3 | | | | | 0.005 |
| *4 | *4a? | | | | | | | | | | | | | | | | 4 | | | | | | | | | | 0.001 |
| *5 | *5a? | | | | | | | | | | | | | | | | | | 5 | | | | | | | | 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. 18 | | Ex. 19 | Int. 19 | Int. 20 | | Ex. 21 | Int. 21 | | Ex. 22 | Ex. 24 | | Int. 24 | | Ex. 26 | | N | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Position | | IVS5 +223 | IVS5 +224 | IVS6 -109 | IVS7 +14 | IVS8 -108 | IVS9 -44 | IVS10 -41 | 1236 | 1342 | IVS12 +17 | IVS13 +24 | IVS13 +81 | IVS14 -38 | 1604 | IVS15 -95 | IVS15 -69 | IVS16 +52 | IVS16 +73 | IVS16 +78 | IVS16 +87 | IVS16 -35 | 2359 | IVS19 -69 | IVS20 +24 | IVS20 -153 | 2677 | IVS21 +49 | IVS21 -72_-76 | 2719 | 2956 | 3043 | IVS24 +18 | 3435 | IVS26 +59 | IVS26 +80 | | |
| Nucleotide change | | A>T | G>T | G>T | G>A | A>G | G>A | T>G | C>T | G>A | G>A | T>C | C>T | G>A | G>A | A>G | T>C | A>C | A>G | T>A | C>T | G>C | C>T | G>A | A>G | G>A | G>T | T>C | GTCT | G>A | A>G | A>G | C>T | C>T | T>G | T>C | | |
| Amino acid change | | | | K140K | | | | | Q412R | E448K | | | | | D630N | | | | | | | | P761W | | | A883T | A883S | | V901I | M986V | T1015A | | I1145I | | | | |
| 1 | *1a | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 117 | 0.110 |
| | *1f | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 47 | 0.044 |
| | *1g | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 27 | 0.025 |
| | *1L | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | 0.009 |
| | *1h | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | 0.006 |
| | *1K | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | 0.006 |
| | *1m | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | 0.003 |
| | *1n | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | 0.003 |
| | *1i | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | 0.002 |
| | *1q | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | 0.002 |
| | *1p | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | 0.002 |
| | Others | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 | 0.005 |
| 2 | *2d | | | | | | | | 2 | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | | | | 382 | 0.368 |
| | *2a | | | | | | | | 2 | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | | | | 7 | 0.007 |
| | *2f | | | | | | | | 2 | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | | | | 4 | 0.004 |
| | *2g | | | | | | | | 2 | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | | | | 3 | 0.003 |
| | *2h | | | | | | | | 2 | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | | | | 3 | 0.003 |
| | *2i | | | | | | | | 2 | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | | | | 1 | 0.001 |
| | *2l | | | | | | | | 2 | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | | | | 1 | 0.001 |
| | *2k | | | | | | | | 2 | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | | | | 1 | 0.001 |
| 4 | *4a | | | | | | | | 4 | | | | | | | | | | | | | | | | | | | | | | | | 4 | | | | 16 | 0.015 |
| | *4c? | | | | | | | | 4 | | | | | | | | | | | | | | | | | | | | | | | | 4 | | | | 1 | 0.001 |
| 8 | *8a | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | | | | 20 | 0.020 |
| | *8b | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | | | | 15 | 0.013 |
| | *8d? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | | | | 1 | 0.001 |
| 8 | *8a | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 98 | 0.092 |
| | *8b | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 17 | 0.016 |
| | *8e | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | 0.009 |
| | *8h | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | 0.009 |
| | *8d | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | 0.003 |
| | *8i | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 3 | 0.003 |
| | *8k | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | 0.002 |
| | *8* | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 0.001 |
| | Others | | | | | | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | 6 | 0.006 |
| 9 | *9b | | | | | | | | 9 | | | | | | | | | | | | | | | | | | 9 | | | | | | | | | | 14 | 0.013 |
| | *9a | | | | | | | | 9 | | | | | | | | | | | | | | | | | | 9 | | | | | | | | | | 7 | 0.007 |
| 10 | *10a | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | | | | | | | | | | 180 | 0.169 |
| | *10e | | | | | | | | | | | | | | | | | | | | | | | | | | 10 | | | | | | | | | | 1 | 0.001 |
| | Others | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 5 | 0.005 |
| 11 | *11a | | | | | | | | 11 | | | | | | | | | | | | | | | | | | 11 | | | | | | | | | | 4 | 0.004 |
| | *11c? | | | | | | | | 11 | | | | | | | | | | | | | | | | | | 11 | | | | | | | | | | 1 | 0.001 |
| 12 | *12a | | | | | | | | | | | | | | 12 | | | | | | | | | | | | | | | | | | | | | | 2 | 0.002 |
| 13 | *13a | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 13 | | | | | | | 2 | 0.002 |
| 14 | *14a? | | | | | | | | | 14 | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 0.001 |
| 15 | *15a? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 15 | | | | | | | 1 | 0.001 |
| 16 | *16a? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 16 | | | | | | 1 | 0.001 |
| 17 | *17a? | | | | | | | | | | | | | | | | | | | | | | 17 | | | | | | | | | | | | | | 1 | 0.001 |
| 18 | *18a? | | | | | | | | | | | | | | | | | | | | | | | | | | 18 | | | | | | 18 | | | | 1 | 0.001 |

**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 | Ex. 27 | Int. 27 | | | | | | | | | | Ex. 28 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | | | I1198S | | | | | | | | | | | G1249G | V1251I | N | Frequency |
| *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.

**Table 3** Diversity of block diplotype combinations across the 4 blocks

| Block −1 | Block 1 | Block 2 | Block 3 | Number of subjects |
|---|---|---|---|---|
| *1a/*1a | *1a/*1a | *2d/*2d | *1a/*1a | 24 |
| *1a/*1a | *1a/*1a | *2d/*2d | *1b/*1a | 13 |
| *1a/*1a | *1c/*1a | *10a/*2d | *1a/*1a | 12 |
| *1a/*1a | *1a/*1a | *2d/*1e | *1b/*1a | 11 |
| *1a/*1a | *1e/*1a | *10a/*2d | *1a/*1a | 9 |
| *1a/*1a | *1a/*1a | *2d/*2d | *1c/*1a | 9 |
| *1a/*1a | *1b/*1a | *8a/*2d | *1a/*1a | 9 |
| *1a/*1a | *1c/*1a | *10a/*1e | *1b/*1a | 6 |
| *1a/*1a | *1g/*1a | *10a/*2d | *1a/*1a | 6 |
| *1a/*1a | *1d/*1c | *10a/*1f | *1a/*1a | 5 |
| *1a/*1a | *2a/*1a | *10a/*2d | *1a/*1a | 5 |
| *1a/*1a | *1c/*1a | *10a/*2d | *1b/*1a | 5 |

A total of 353 diplotype-combinations across the 4 blocks were detected in 533 subjects.
The number of subjects for the other combinations was less than 5.

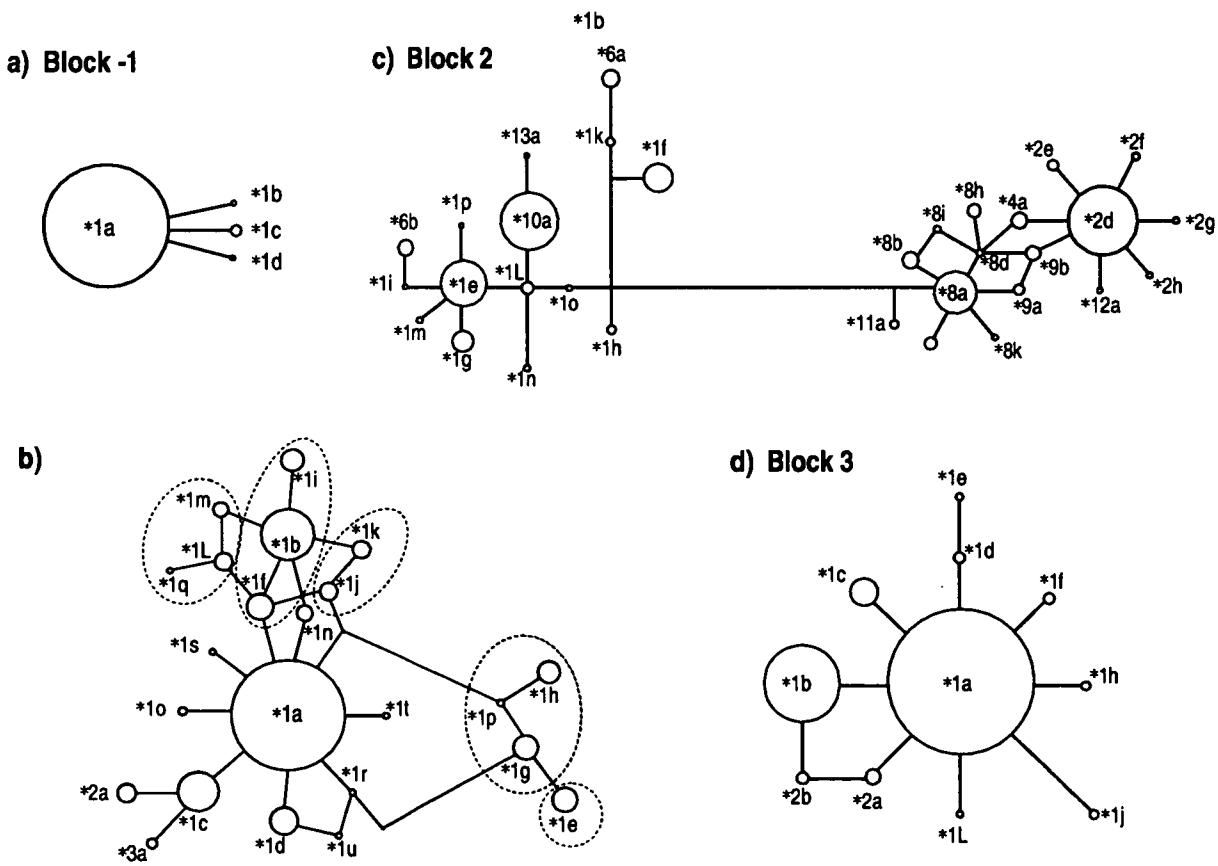



**Figure 6** Network analysis of *ABCB1* haplotypes of Block −1 (a), Block 1 (b). Block 2 (c) and Block 3 (d). For each block, the circle area represents the approximate haplotype frequency, and line length between the circles is proportional to the number of mutations. Haplotypes inferred in only one chromosome were omitted from this analysis. The classification by Takane *et al.* (2004) is indicated with dashed lines.

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 additional 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_-1457delCATCC.

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.

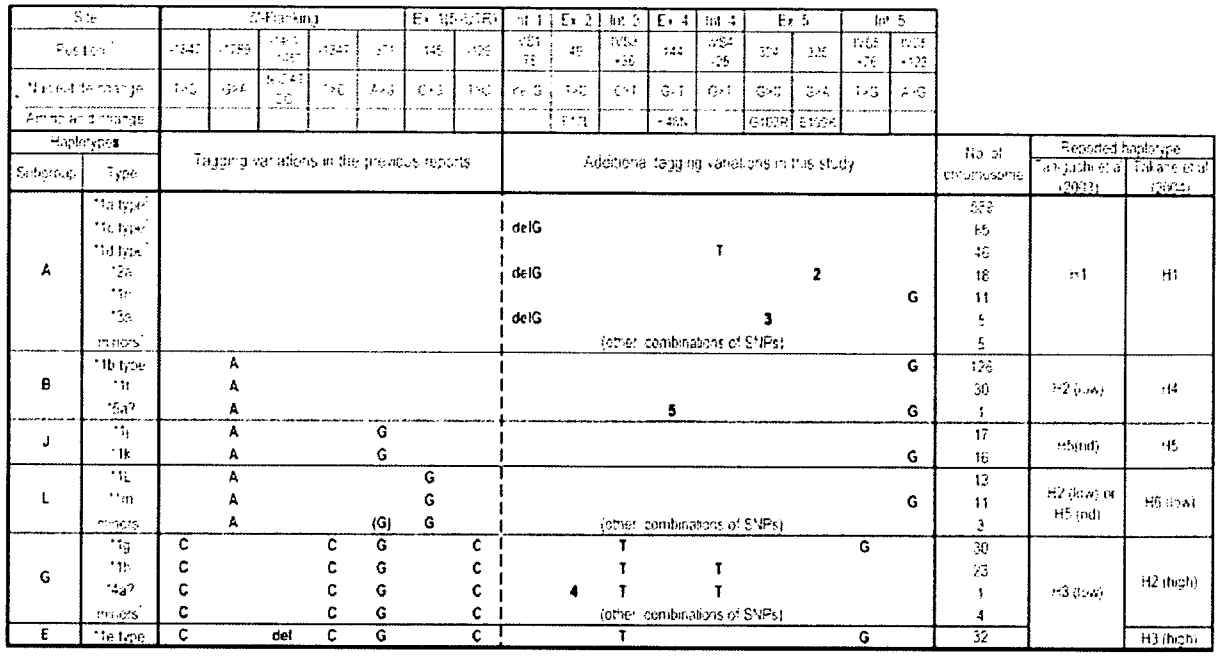


**Figure 7** New classification of Block 1 haplotypes and comparison with reported promoter region haplotypes. Genetic variations (allele frequency >0.01) and nonsynonymous variations in Block 1 were sorted according to marker variation, and classified into 6 subgroups (A, B, J, L, G and E).

[a]The positions in other reports were adjusted to the nucleotide numbers used in this study.

[b]The *1a type includes *1a, *1o, *1s, *1t, *1v, *1w, *1x, *1y.

[c]The *1c type includes the *1c haplotype and an ambiguously defined *1 haplotype.

[d]The *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.

[f]The *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.

[i]The *1e type includes the *1e haplotype and one ambiguously defined *1 haplotype.

[j]Altered 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 diplotypes 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.