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The Promoter Region of the *MDR1* Gene Is Largely Invariant, but Different Single Nucleotide Polymorphism Haplotypes Affect MDR1 Promoter Activity Differently in Different Cell Lines

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ABSTRACT

The MDR1 multidrug transporter represents one of the better characterized drug transporters that play an important role in protecting the body against xenobiotic insults. Single nucleotide polymorphisms (SNPs) and SNP haplotypes within this gene have been variously associated with differences in MDR1 expression/function, drug response as well as disease susceptibility. Nonetheless, the effect of polymorphisms at the *MDR*1 promoter region on its promoter activity remains less characterized. Through the examination of ~1.5 kilobases of MDR1 promoter region from five populations, including the Chinese, Malays, Indians, European Americans, and African Americans, we identified eight low-frequency SNPs, of which only two were polymorphic in at least four of the five populations examined. The other SNPs are mainly population-specific, the majority of which occur only in the African-American population. Recapitulation of the various combinations of SNP haplotypes in vitro in promoter-reporter assays revealed a few notable trends. The African and European American-specific haplotypes tended to result in enhanced MDR1 promoter activity only in the human embryonic kidney (HEK) 293 cell line. Haplotype GCTAACC, which occurs at variable frequencies in all the populations examined, with Asians having much lower frequencies (<2%) compared with the European Americans/African Americans (>4%), affected MDR1 promoter activity differently in different cell lines. Compared with the commonest haplotype, GCTA-ACC haplotype resulted in a significant decrease in MDR1 promoter activity in HeLa cells ($P < 0.05$) but a significant increase in the same promoter activity in HEK293 cells ($P < 0.05$). These results suggest that the MDR1 promoter region is largely invariant but that different haplotypes have differential effects on the MDR1 promoter activity in different cell lines.

Initially discovered as an important player in multidrug resistance during cancer chemotherapy, the MDR1 multidrug transporter regulates the traffic of a variety of diverse drugs/xenobiotics, including antiarrhythmics, antidepressants, antipsychotics, and antivirals across biological membranes (for reviews, see Lee et al., 2004a; Marzolini et al., 2004). Also known as P-glycoprotein, the MDR1 transporter is a 170-kDa cell surface phosphoprotein comprising 1280 amino acids. This protein contains two homologous halves, each traversing the plasma membrane six times with one ATP-binding site. In the last few years, unprecedented scientific attention has been focused on SNPs within the *MDR1* gene and its association with MDR1 expression, function, and drug response (Lee et al., 2004a; Marzolini et al., 2004). *MDR1* SNPs, particularly exonic SNPs, have also been associated with susceptibility to renal epithelial tumor (Siegsmund et al., 2002); ulcerative colitis and Crohn's disease (Schwab et al., 2003); Parkinson's disease (Furuno et al., 2002; Drozdzik et al., 2003; Lee et al., 2004b; Tan et al., 2004, 2005); human immunodeficiency virus-1 infection (Ifergan et al., 2002); and other diseases (Marzolini et al., 2004). SNPs within the MDR1 promoter region, which may play an important role in the regulation of *MDR1* gene expression, have thus

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ABBREVIATIONS: MDR, multidrug resistance; SNP, single nucleotide polymorphism; kb, kilobase(s); TLSS, translational start site; bp, base pair(s); EGFP, enhanced green fluorescent protein; CMV, constitutive cytomegalovirus; HEK, human embryonic kidney; CPRG, chlorphenol red-β-D-galactopyranoside; TSS, transcription start site(s); CEPH, Centre d'Etude du Polymorphisme Humain.

far been examined only in two reports (Taniguchi et al., 2003; Takane et al., 2004).

Two major transcription start regions and two promoters separated by approximately 100 kb were identified in the *MDR1* gene (Fig. 1A) (Ueda et al., 1987a,b; Gottesman and Pastan, 1993). The proximal promoter was found to drive the expression of most MDR1 transcripts found in normal tissues, including the liver, kidney, and adrenal gland, whereas the distal promoter, which is still undefined and believed to be a cryptic promoter, was found to drive MDR1 expression in colchicine-selected cell lines (Ueda et al., 1987a; Gottesman and Pastan, 1993), mononuclear cells of patients with acute lymphoblastic leukemia who overexpress the MDR1 transporter (Rothenberg et al., 1989) as well as primary breast tumors (Raguz et al., 2004). In this study, we will only focus on the proximal promoter.

The proximal promoter of the *MDR1* gene is TATA-less and contains two transcription start sites, a CAAT box, two GC boxes, and several important transcription binding sites regulating its expression in response to external stimuli (Labialle et al., 2002). Cytotoxic drugs (Kohno et al., 1989; Stein et al., 1996), serum starvation (Tanimura et al., 1992), hypoxia (Comerford et al., 2002), heat shock (Chin et al., 1990; Miyazaki et al., 1992; Vilaboa et al., 2000), and UV irradiation (Uchiumi et al., 1993; Ohga et al., 1996; Hu et al., 2000) are some of the factors reported to modulate *MDR1* expression and promoter activity. The Y-box containing an inverted CCAAT sequence (Uchiumi et al., 1993; Ohga et al., 1996), CCAAT, and the proximal GC element (Hu et al., 2000) have been implicated in the regulation of MDR1 promoter activity in response to UV irradiation.

SNPs at the promoters of various genes have been shown to affect promoter activity as well as associate with differences in its gene expression and susceptibility to diseases (Joosten et al., 2001; Aklillu et al., 2003; Sibley et al., 2003). Because the MDR1 transporter potentially protects the individual against environmental stress and is a significant determinant of the success of cancer chemotherapy or other drug therapy, SNPs at its promoter may influence its gene expression and account for differences in response to drug therapy or susceptibility to various diseases between individuals. There are two reports examining SNPs and haplotype of SNPs at the *MDR1* promoter locus in the Japanese population (Taniguchi et al., 2003; Takane et al., 2004). Both studies examined SNP haplotypes in slightly different 2-kb region of the MDR1 promoter and identified different relatively low-

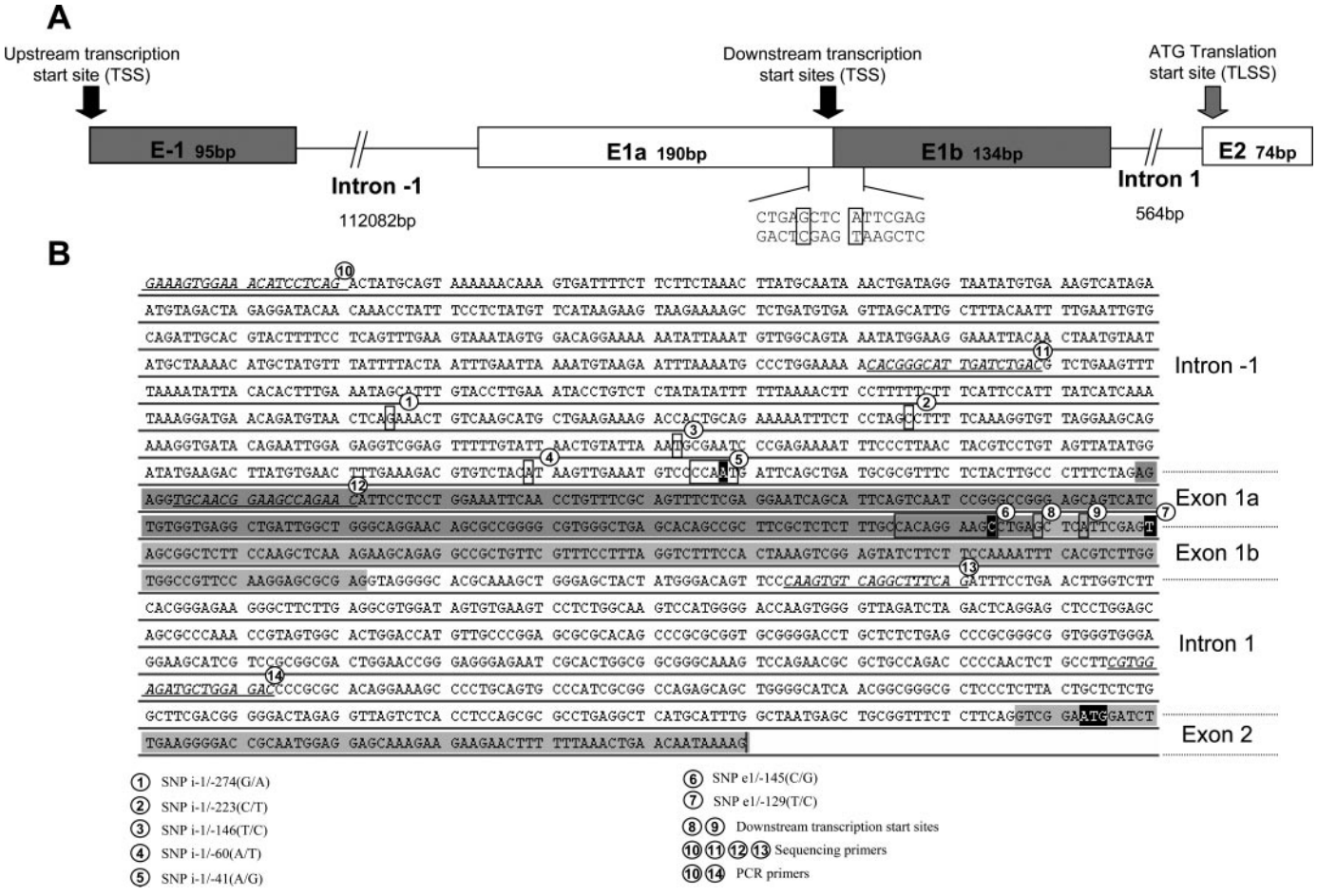

Fig. 1. Structure and sequence of the *MDR1* promoter. A, diagrammatic depiction of region upstream the TLSS of the *MDR1* gene. Two transcription start regions denoted as distal and proximal transcription start regions are shown. Transcript arising from the distal transcription start region include exon-1 (E-1) and exon 1a (E1a), whereas transcripts arising from the proximal transcription start region begin with exon 1b (E1b). The proximal transcription start region contains at least two major TSS as highlighted within the sequence context. The TLSS resides in exon 2. B, sequence of proximal *MDR1* promoter region was obtained from GenBank (AC022457). The SNPs examined in detail in this study are highlighted as 1 to 7. The two major TSS at the proximal transcription start region are numbered 8 and 9. Location of primers used for amplification of the *MDR1* promoter is indicated by 10 and 14.

frequency polymorphisms with slight allelic differences in promoter activity (Taniguchi et al., 2003; Takane et al., 2004). In this study, we scanned for polymorphisms in a 1.5-kb region of the MDR1 promoter in five different populations and characterized the effect of SNP haplotypes on MDR1 promoter activity.

Materials and Methods

Genomic DNA from Study Populations. Genomic DNA was isolated from discarded umbilical cord blood of ~96 random, anonymous neonates from each of the three major ethnic populations in Singapore (Chinese, Malay, and Indian). All original identifiers, except ethnicity, have been removed and destroyed before the isolation of DNA. These studies fall within the guidelines as spelled out in our IRB guidelines [NUS_IRB guidelines (IRB-GUIDE-006 #4 and OHRP Guidelines 45 CFR 46.101] and the Human Tissue Research report of the Bioethics Advisory Committee (part IV, section 8, paragraph 8.10), and exemption from IRB review was thus obtained from our institution (NUS-IRB Reference Code 04-126E). Genomic DNA samples from European Americans and African Americans were purchased from the respective Human Variation Collections in the National Institute of General Medical Sciences Human Genetic Cell Repository (The Coriell Institute for Medical Research, Camden, NJ).

Identification of MDR1 Promoter SNPs. To identify promoter SNPs at the *MDR1* gene, 1.5 kb of DNA from 180 bp upstream of the translational start site (TLSS) (including intron1, exon 1a and b, and part of intron −1) was amplified using 4 ng/μl genomic DNA, 0.4 mM dNTPs, 0.04U HotStar *Taq* polymerase (QIAGEN GmbH, Hilden, Germany) and 0.2 pmol/μl of each of the following primers 5′-*GAGA*GAATTCGAAAGTGGAAACATCCTCAG-3′ (forward primer) and 5′-*CTCT*GTCGACGTCTCCAGCATCTCCACG-3′ (reverse primer) (Fig. 1B). Underlined sequences are EcoRI and SalI restriction sites, respectively, to facilitate cloning of this promoter into a promoter-reporter construct. Sequences in italics are buffer sequences to facilitate restriction enzyme digestion. Amplification conditions include an initial denaturation at 94°C for 15 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 2 min followed by a final extension at 72°C for 5 min. Dideoxy sequencing was performed on the ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA) and CEQ 8000 genetic analysis system (Beckman Coulter, Fullerton, CA) using the following primers: 5′-GAAAGTGGAAACATCCTCAG-3′, 5′-CACGGGCATTGATCTGAC-3′, 5′-TGCAACGGAAGCCAGAAC-3′, and 5′-CAAGTGTCAGGCTTCAG-3′. Allele, genotype, and haplotype frequencies were evaluated as described previously (Tang et al., 2004).

Functional Characterization of MDR1 Promoter SNPs. DNA (1.5 kb) from 180 bp upstream of the TLSS representing the *MDR1* promoter was amplified using primers as described above and cloned into a promoter-reporter construct (Fig. 3A). SNP haplotypes were recapitulated using PCR mutagenesis (Fig. 3B), digested with EheI and BglII, and cloned into the promoter-reporter construct (Fig. 3A). All plasmid constructs containing normal and variant *MDR1* promoters were sequenced across the PCR amplified regions to exclude PCR-induced nucleotide misincorporations before use. The promoter-reporter construct comprises the β-galactosidase reporter gene driven by the MDR1 (or its variants) promoter as well as the enhanced green fluorescent protein (EGFP) driven by the constitutive cytomegalovirus (CMV) promoter to normalize for differences in transfection efficiencies. The various constructs were transfected using calcium phosphate coprecipitation as described previously (Lee et al., 2000) into either human epithelial cervical adenoma (HeLa); KB3-1, a subclone of HeLa with undetectable *MDR1* expression; or human embryonic kidney (HEK) 293 cell lines. Forty-eight hours after transfection, the cells were harvested for reporter gene assays. β-Galactosidase activity was assayed kinetically using chlorphenol red-β-D-galactopyranoside (GPRG) as substrate and measured at 1-min intervals over 60 min at 570 nm in a SpectraMAX PLUS microplate reader (Molecular Devices, Sunnyvale, CA). Western blot analyses were performed using 0.02 μg/ml mouse anti-green fluorescent protein (Roche Diagnostics, Indianapolis, IN) and 1:100,000 horseradish peroxidase-conjugated goat anti-mouse IgG (Pierce Chemical, Rockford, IL) antibodies to quantitate the EGFP protein levels. β-Galactosidase expression was normalized against EGFP protein levels.

Results

The Promoter of the *MDR1* Gene Has Only a Few Low Minor Allele Frequency SNPs. Eight SNPs occurring in at least one population were identified from 1.5 kb of DNA 180 bp upstream the TLSS of the *MDR1* gene (Table 1). The frequencies of the minor alleles of all the identified SNPs were approximately 10% or less. Notably only SNPe1/-129(T/C) is polymorphic in all five populations, and SNPi1/-41(A/G) is polymorphic in four of five populations examined. The other SNPs occur at very low frequencies (<4%) and were population-specific, most of which were only polymorphic in the African-American population except for SNPe1/-145, which occurred only in the Chinese population, and SNPi1/-223(C/T), which is specific to the European American population. Pairwise comparison of SNP allele frequencies between populations revealed statistical difference in SNPi1/60(A/T) between the African American and the other four populations and in SNPi1/-41(A/G) between the Chinese/Malay and the other three populations (Table 1). For SNPe1/-129(T/C), the Indian population is significantly different from the other four populations. These data highlight the largely invariant and population-specific nature of SNPs at the MDR1 promoter locus. We proceeded to characterize the haplotypes of polymorphisms of SNPs and their functional significance in vitro using a promoter-reporter assay.

Only a Single Major Haplotype Occurring at Frequencies of 85% or Greater Is Represented in All Five Populations. Of 128 possible haplotypes from the seven SNPs examined at the MDR1 promoter locus, only between two to six haplotypes, inferred by the Expectation-Maximization algorithm, were observed in the five different populations (Fig. 2). The most common haplotype, GCTAACT, occurs in all population at frequencies greater than 85%. The only other haplotype that occurs in all population is the GCTAAC**C** haplotype, but its frequency is low and variable with the Asian populations having a much lower (<2%) frequency compared with the European-American/African-American populations (>4%). The closely related haplotype (GCTA**G**C**C**) was observed at low frequencies in four of the five populations examined. It is noteworthy that, in contrast to the GCTAAC**C** haplotype, the frequency of GCTA**G**C**C** is higher in the Asian populations (Chinese and Malays) (>3%) compared with European American and African Americans (<1%). The Indian ethnic group has the fewest number of haplotypes (two) as it is monomorphic at two SNP loci [i1/-41(A/G) and e1/-145(C/G)].

Polymorphisms within the MDR1 Promoter Influences Its Activity Differently in Different Cell Lines. To evaluate the effect of these SNPs on the MDR1 promoter activity, we recapitulated these SNPs in vitro using PCR mutagenesis (Fig. 3B) and cloned them into the promoter-

TABLE 1

Single nucleotide polymorphisms identified in 1.5-kb region of the MDR1 promoter

Bold values represent statistical difference (at P value indicated) between the population indicated in the row and the population indicated in the column.

SNP no.	SNP ID	Population	n	HWE P Value	Allele Frequency				Pairwise Differences Fisher's Exact P Value				
					A	C	T	G	CH	ML	IN	EA	AA
					%								
1	I-1/-274 (G/A)	CH	89	1.00	0.0			100.0		1.00	1.00	1.00	0.50
		ML	86	1.00	0.0			100.0			1.00	1.00	0.50
		IN	88	1.00	0.0			100.0				1.00	0.50
		EA	95	1.00	0.0			100.0					0.23
		AA	89	0.91	1.1			98.9					
2	I-1/-223 (C/T)	CH	89	1.00		100.0	0.0			1.00	1.00	1.00	1.00
		ML	86	1.00		100.0	0.0				1.00	1.00	1.00
		IN	88	1.00		100.0	0.0					1.00	1.00
		EA	95	0.96		99.5	0.5						1.00
		AA	89	1.00		100.0	0.0						
3	I-1/-146 (T/C)	CH	89	1.00		0.0	100.0			1.00	1.00	1.00	1.00
		ML	86	1.00		0.0	100.0				1.00	1.00	1.00
		IN	88	1.00		0.0	100.0					1.00	1.00
		EA	95	1.00		0.0	100.0						0.48
		AA	89	0.96		0.6	99.4						
4	I-1/-60 (A/T)	CH	89	1.00	100.0		0.0			1.00	1.00	1.00	**0.01**
		ML	86	1.00	100.0		0.0				1.00	1.00	**0.01**
		IN	88	1.00	100.0		0.0					1.00	**0.01**
		EA	95	1.00	100.0		0.0						**5.82×10^{-3}**
		AA	89	0.02	96.1		3.9						
5	I-1/-41 (A/G)	CH	89	0.20	89.9			10.1		0.11	**4.87×10^{-6}**	**1.64×10^{-5}**	**5.09×10^{-5}**
		ML	86	0.61	94.8			5.2			**1.58×10^{-3}**	**7.86×10^{-3}**	**9.56×10^{-3}**
		IN	88	1.00	100.0			0.0				1.00	1.00
		EA	95	0.96	99.5			0.5					1.00
		AA	89	0.96	99.4			0.6					
6	E1/-145 (C/G)	CH	89	0.96		99.4		0.6		1.00	1.00	0.48	1.00
		ML	86	1.00		100.0		0.0			1.00	1.00	1.00
		IN	88	1.00		100.0		0.0				1.00	1.00
		EA	95	1.00		100.0		0.0					1.00
		AA	89	1.00		100.0		0.0					
7	e1/-129 (T/C)	CH	89	0.66		4.5	95.5			0.81	**0.04**	1.00	0.49
		ML	86	0.61		5.2	94.8				**9.9×10^{-3}**	1.00	0.65
		IN	88	0.96		0.6	99.4					**0.02**	**2.91×10^{-3}**
		EA	95	0.62		4.7	95.3						0.50
		AA	89	0.32		6.7	93.3						
8	E1/-43 (A/G)	CH	89	1.00	100.0			0.0		1.00	1.00	1.00	0.12
		ML	86	1.00	100.0			0.0			1.00	1.00	0.12
		IN	88	1.00	100.0			0.0				1.00	0.12
		EA	95	1.00	100.0			0.0					0.05
		AA	89	0.83	97.8			2.2					

reporter constructs whereby the *MDR1* (or its variant) promoter drives the β-galactosidase reporter gene and the constitutive CMV promoter drives the EGFP gene for normalization of transfection efficiency (Fig. 3A). As shown in Fig. 3C, a few notable observations were made on the effect that the different haplotypes have on the MDR1 promoter activity in the different cell lines. Four haplotypes which occur specifically in either the European American (G**T**TAACT) or African American (**A**CTAACT, GC**C**AACT, GC**T**ACT) (Fig. 2) were found to mediate approximately 2-fold greater MDR1 promoter activity in HEK293 cell-line than the most common GCTAACT haplotype although only **A**CTAACT and GC**C**AACT haplotypes showed statistically different promoter activity compared with the most common haplotype (Fig. 3C). It is odd that the reverse trend was observed in HeLa cell-line where these four haplotypes displayed approximately 2-fold lower MDR1 promoter activity compared with the most common GCTAACT haplotype although no statistical differences were observed (Fig. 3C). No differences in MDR1 promoter activity were observed for any of the four haplotypes in KB3-1 cell line, which does not support MDR1 expression (Fig. 3C). The GCTA**G**CT haplotype which occurs only in the Southeast Asian population (Chinese and Malays) does not seem to change MDR1 promoter activity in all three cell-lines examined. The GCTAA**G**T haplotype, which was not found in the five populations examined was found to significantly reduce MDR1 promoter activity ($P < 0.05$) by more than 3-fold in HeLa cell line but did not significantly affect MDR1 promoter activity in KB3-1 or HEK293 cell lines (Fig. 3C). An interesting observation was made on the GCTAAC**C** haplotype, which occurs at variable frequencies in all the populations examined, with Asians having much lower frequencies (<2%) compared with the European Americans/African Americans (>4%) (Fig. 2). This haplotype resulted in statistically significant ($P < 0.05$) enhancement in MDR promoter activity in HEK293 cells but statistically significant ($P < 0.05$) attenuation of the promoter activity in HeLa cells and no statistically significant difference in promoter activity in KB3-1 cells (Fig. 3). Haplotype GCTA**GG**T, representing a combination of the GCTA**G**CT haplotype, which does not significantly affect MDR1 promoter activity, and GCTAA**G**T, which significantly reduced MDR1 promoter activity in HeLa cells, is specific only to the Chinese population and displays promoter activity that is intermediate between the two haplotypes and not statistically different from the most common GCTAACT haplotype. The haplotype GCTA**G**C**C,** which is a combination of GCTA**G**CT (no effect on MDR1 promoter activity) and GCTAAC**C** haplotypes (significantly reduced MDR1 promoter activity in HeLa cells but significantly enhanced promoter activity in HEK293 cells), occurs in all populations examined except the Indian population, also displayed promoter activity that is not statistically significant and intermediate between these haplotypes (Fig. 3C). Haplotype GCTAA**GC**, which represents a combination of GCTAA**G**T and GCTAAC**C** haplotypes, both of which significantly decreased MDR1 promoter activity in HeLa cells, was not found in any of the five populations examined but displayed an even greater attenuation of MDR1 promoter activity than either GCTAA**G**T and GCTAAC**C** haplotype alone, suggesting an additive effect of promoter activity of the two individual SNPs (Fig. 3C). Likewise, when all three SNPs were the minor alleles in the haplotype GCTA**GGC**, the overall MDR1 promoter activity is still significantly lower than the most common haplotype GCTAACT but not as low as either the GCTAA**G**T or

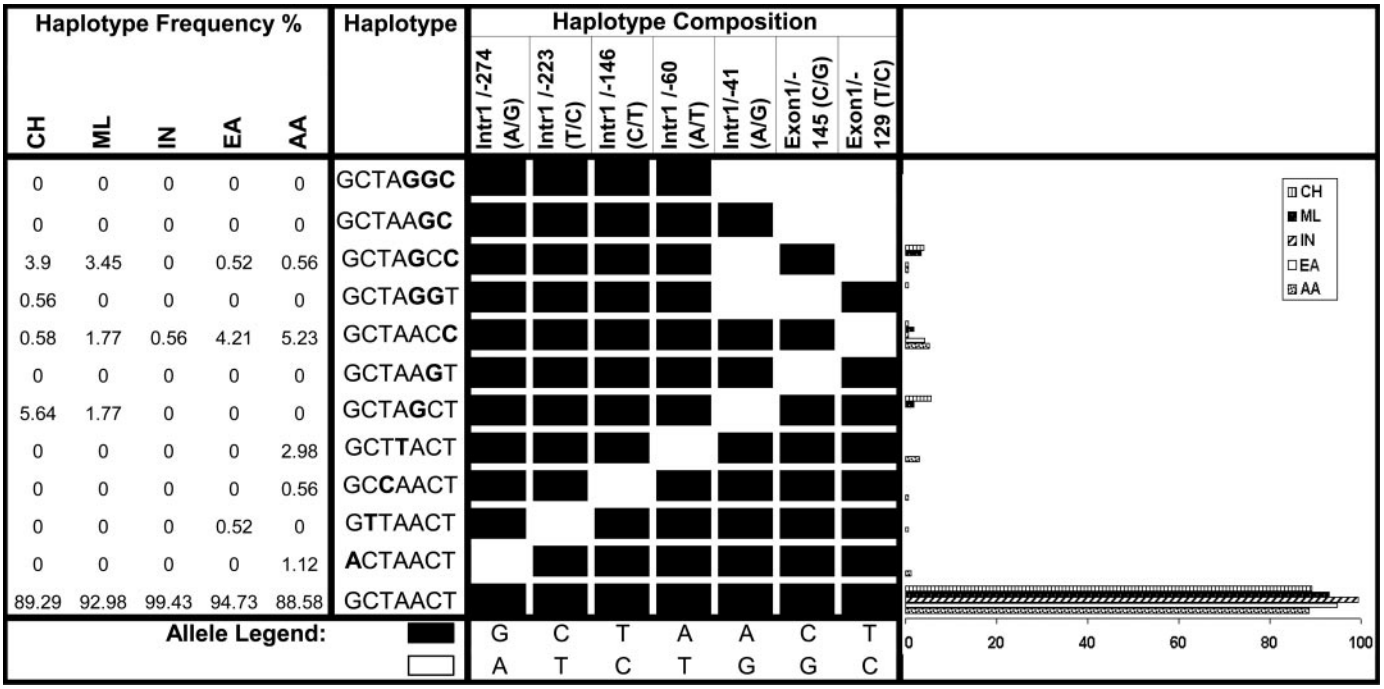

Fig. 2. Haplotype profile at the *MDR1* promoter locus. The actual value of the haplotype frequency (percentage) in the different populations is shown in the left-most panel (CH, Chinese; ML, Malay; IN, Indian; EA, European American; and AA, African American) The haplotype identity is shown on the right of haplotype frequency panel. The actual SNP composition of the haplotype is indicated in the panel on the right of the haplotype (black box represents the major SNP allele, and white box represents the minor allele). Graphical representation of the haplotype frequencies in the different populations is shown at the right-most panel.

GCTAAC**C** haplotype alone, again suggesting additive effect of promoter activities from the three SNPs (Fig. 3C).

Together, these results suggest the following properties of polymorphisms at the MDR1 promoter region. First, all the polymorphisms observed in this promoter region are of low frequency (<5%). Second, the distribution of polymorphisms that functionally affect MDR1 promoter activity is different in the various populations with Chinese sharing more common allele frequencies, with the Malays and the European American sharing more common allele frequencies with the African Americans. Indeed, some of these polymorphisms are population-specific. Third, the same haplotype can have opposite effects on the MDR1 promoter activity depending on the cell line into which it is introduced. Finally, the MDR1 promoter activity of cells containing a haplotype in which two or more sites carry the minor allele will reflect the additive effect of the combination of promoter activities from haplotypes that carry each of the minor allele alone.

Discussion

Because the MDR1 transporter regulates the transport of diverse spectrum of drugs and xenobiotics and polymorphisms within the promoter region of the gene may alter its intrinsic promoter activity and hence expression of the *MDR1* gene, we scanned for polymorphisms within a region of ~1.5 kb from ~180 bp upstream the TLSS of the *MDR1* gene (Fig. 1B) in ~85 to 95 individuals from five different populations. Eight SNPs, all of which occurred at frequencies of ≤10%, were identified in at least one population (Table 1). Except for SNPs i1/-41(A/G) and e1/-129(T/C), which were polymorphic in all or four of the five populations examined, the other SNPs were population-specific; most of the SNPs were African-American-specific except for SNPs i1/-223(A/G) and e1/-129(T/C), which were polymorphic only in the European-American and Chinese populations, respectively (Table 1). We also examined MDR1 promoter SNPs from the public

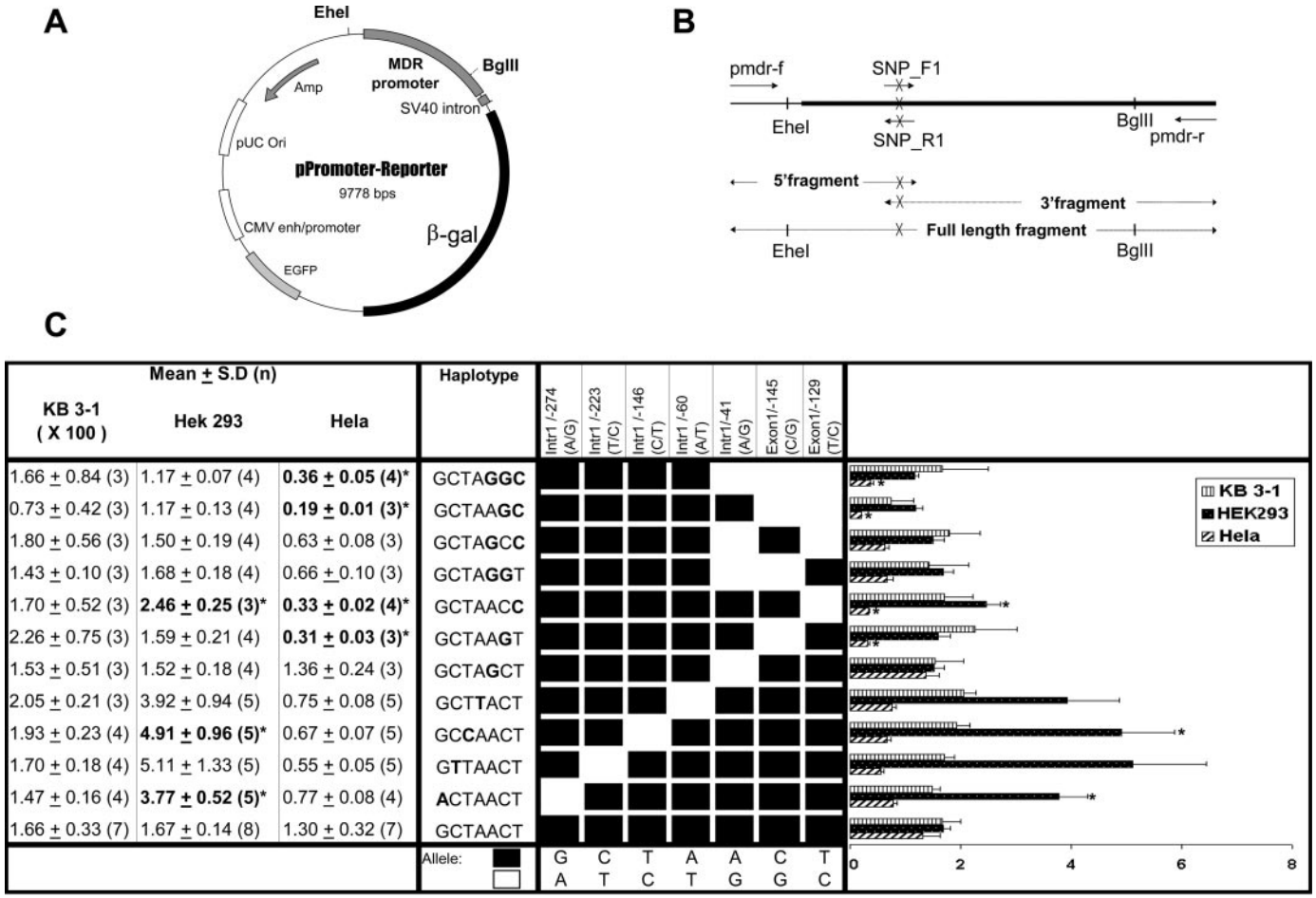

Fig. 3. Effect of MDR1 promoter SNPs on its promoter activity. A, promoter-reporter construct for the cloning of the MDR1 promoter and its variants. In this construct, the MDR1 promoter (or its variants) drives the β-galactosidase gene, whereas the CMV promoter drives the EGFP gene to normalize for differences in transfection efficiency. B, a two-step PCR mutagenesis strategy was used for the in vitro recapitulation of SNPs or haplotype of SNPs at the MDR1 promoter region. In the first step, two PCR products was generated using primers pmdr-f and SNP_R1(containing the reverse complement of the variant) as well as pmdr-r and SNP_F1 (containing the variant). Equal molar amounts of product from these two PCRs are mixed, and another PCR is performed using primers pmdr-f and pmdr-r to generate the variant. C, effect of SNP haplotypes on the MDR1 promoter activity in KB3-1, HEK293, and HeLa cells. Leftmost panel shows the values of the mean, S.D., and the number of replicate experiments (n) of the β-galactosidase reporter activity in the three different cell lines. Promoter activity is expressed as β-galactosidase activity normalized against EGFP fluorescence ($OD \cdot s^{-1}_{\beta\text{-gal}} / RFU_{egfp}$). The reporter activity for KB3-1 cells was multiplied by 100 because its activity was much lower than the other two cell lines. Bold numbers with asterisk (*) represents reporter activities that were statistically different ($P < 0.05$) from the most common haplotype (GCTAACT). The haplotype identity is shown on the right of β-galactosidase reporter activity panel. The actual SNP composition of the haplotype is indicated in the panel on the right of the haplotype (black box represents the major SNP allele, and white box represents the minor allele). Graphical representation of the β-galactosidase reporter activities in the three different cell lines is shown at the right-most panel. Asterisk (*) denotes statistical difference at $P < 0.05$.

databases, HAPMAP (http://www.hapmap.org/), which evaluated 60 samples from each of the Chinese, Japanese, CEPH, and Yoruba populations, and Perlegen (http://genome.perlegen.com/browser/index.html), which analyzed 24 samples from each of the Chinese, European-American, and African-American populations. These databases revealed that only SNPs i1/-41(A/G) and e1/-129(T/C) were genotyped. Although the genotype frequencies of SNPe1/ -129(T/C) from the two databases were similar to each other and to our results, the frequency of SNPi1/-41(A/G) in the European American/CEPH and African American/Yoruba were slightly different in the different studies. This is not unusual because SNPi1/41(A/G) occurred at very low frequency and thus may not be identified in every sampling of the population, especially if the numbers of individuals sampled were low. Sequencing of the core promoter region in another European population was reported to identify only SNP e1/-129T>C where the minor C-allele only occurs at a frequency of 5.9% (Hoffmeyer et al., 2000). In the Japanese population, various groups using either sequencing or single strand conformation polymorphism also identified SNPs i1/ -41(A/G), e1/-129(T/C), and sometimes SNPe1/-145(C/G) at the MDR1 promoter region (Ito et al., 2001; Tanabe et al., 2001; Saito et al., 2002; Taniguchi et al., 2003; Takane et al., 2004). The minor allele frequency of these SNPs in the Japanese population was reported to be ≤10% (Table 2; Lee et al., 2004a).

Two Japanese groups (Taniguchi et al., 2003; Takane et al., 2004) using either sequencing or PCR-single strand conformation polymorphism screened between 2 and 4 kb of the MDR1 promoter in Japanese (Taniguchi et al., 2003) or Japanese and Caucasian (Takane et al., 2004) populations. Only SNPs i1/-41(A/G), e1/-129(T/C), and i1/-1459(G/A) were similar to the SNPs we identified. Except for SNPs i1/-432(A/G) and i1/-755(A/G), all the other SNPs were beyond the region that we examined. These two SNPs were found not to be polymorphic in any of the populations we examined. It is noteworthy that the two groups identified different polymorphisms from similar DNA region in the same Japanese population, probably because of low frequencies of these polymorphisms. Taniguchi et al. (2003) but not Takane et al. (2004) found SNPs i1/-755(A/G) and i1/-432(A/G) to be polymorphic in the Japanese population. Likewise, Takane et al. (2004) but not Taniguchi et al. (2003) found SNPs i1/-824(T/C) and e1/-145(C/G) to be polymorphic in Japanese. Except for SNP i1/-1459(G/A), which resides far from the core promoter and has a frequency of ~25 to 28%, the frequencies of all the SNPs that both groups identified at the *MDR1* promoter were also ≤10%.

A search through the major public SNP databases also did not reveal additional SNPs occurring at frequencies higher than 10% at the *MDR1* promoter. This is unlike SNPs at the promoter of a closely related MRP1 drug transporter where the minor allele frequency of the positively selected promoter SNP [5′FR/-260(G/C)] can be as high as 55% in a population (Wang et al., 2005). Together, these data suggest that the core promoter region of the *MDR1* gene is primarily invariant with only a few low-frequency SNPs, many of which are population-specific.

A recent report which examined the distribution of SNPs at ~2-kb promoter regions of genes within the human genome in silico (Guo and Jamison, 2005) found that more SNPs reside close to the transcription start sites (TSS) of genes with a disproportionately greater number of SNPs resulting from G/C nucleotide transversions. However, it seems that the distribution of SNPs at the MDR1 promoter is quite unlike what was described above for the other genes in the human genome (Table 2). Only two relatively low-frequency SNPs (≤10%) that occur in at least four different populations were found near the TSS of the MDR1 promoter. The other SNPs identified within 1 kb of the TSS were of very low frequency (<4%) and primarily population-specific. Three SNPs with frequencies of between 8 and 28% identified in the Japanese population reside beyond 1 kb from the TSS (Table 2) (Taniguchi et al., 2003; Takane et al., 2004). In addition, unlike SNPs in other promoter regions whereby more SNPs result from G/C nucleotide transversions, all but two SNPs at the promoter region of the MDR1 promoter resulted from transition changes. Of the two transversion SNPs, which are population-specific and of very low frequency (≤4%), only SNPe1/-145(C/G) is a G/C transversion. Hence, it seems that the MDR1 promoter is largely invariant and atypical.

To evaluate the functional significance of these SNPs, we recapitulated these SNP-haplotypes in vitro and evaluated the *MDR*1 promoter activity. We observed two interesting trends (Fig. 3). First, the same haplotype can have differential effects on different cell types/lines. As evident in Fig. 3, the GCTAAC**C** haplotype, which occurs at variable frequencies in all the populations examined, with Asians having much lower frequencies (<2%) compared with the European Americans/African Americans (>4%) (Fig. 2), resulted in statistically significant ($P < 0.05$) enhancement in MDR1 promoter activity in HEK293 cells but statistically significant ($P < 0.05$) attenuation of the promoter activity in HeLa cells and no statistically significant difference in promoter activity in KB3-1 cells (Fig. 3). In addition, population-specific haplotypes, which occur only in either the European Americans (G**T**TAACT) or African Americans (**A**CTAACT, GC**C**AACT, and GCT**T**ACT) (Fig. 2), were found to result in higher mean MDR1 promoter activity in HEK293 cells but lower mean MDR1 promoter activity in HeLa cells than the most common GCTAACT haplotype, although only **A**CTAACT and GC**C**AACT haplotypes showed statistically different promoter activity compared with the most common haplotype (Fig. 3C). No differences in MDR1 promoter activity were observed for any of the four haplotypes in KB3-1 cell line, which does not support MDR1 expression (Fig. 3C). Second, the effect of more than one polymorphism in a single haplotype is additive. For example, the haplotype GCTA**G**C**C,** which represents the combination of GCTA**G**CT (no effect on MDR1 promoter activity) and GCTAAC**C** haplotypes (significantly reduced MDR1 promoter activity in HeLa cells but significantly enhanced promoter activity in HEK293 cells), displayed promoter activity that is not statistically significant and intermediate between these haplotypes (Fig. 3C). Similar observations were made for the other haplotypes carrying more than one polymorphisms (Fig. 3).

Two other Japanese groups also examined promoter-reporter activities of some haplotypes of SNPs at the MDR1 promoter region in HepG2 cells (Taniguchi et al., 2003; Takane et al., 2004). Taniguchi et al. (2003) examined three different haplotypes making up five SNPs in a region of ~2 kb from ~560 bp upstream from the TLSS, whereas Takane et al. (2004) examined 10 haplotypes encompassing 10 SNPs

TABLE 2
Comparison of the frequency of SNPs at the MDR1 promoter in the different databases or reports

	SNP ID	rs #	Region	Putative Transcription Binding Sites		Population	This Study (n~86-95) Allele frequency %		HAPMAP (n~60) Allele frequency %		Perlegen (n~25) Allele frequency%		Takane *et al* (2004) (n~94-96) Allele frequency %		Taniguchi *et al* (2003) (n~25) Allele frequency %	
				C	T		C	T	C	T	C	T	C	T	C	T
1	i1 /-2010(T/C)		Intron 1	--	Zebrafish PAX2 paired domain protein	CH	--	--	--	--	--	--	--	--	--	--
						ML	--	--	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	--	--	2.00	98.00
						IN	--	--	--	--	--	--	--	--	--	--
						EA / Ceph	--	--	--	--	--	--	--	--	--	--
						AA / YRI	--	--	--	--	--	--	--	--	--	--
				C	T		C	T	C	T	C	T	C	T	C	T
2	i1/-1517(T/C)		Intron 1	COMP1, cooperates with myogenic proteins in multicomponent complex	Abd-B-like homeodomain protein Hoxb-9	CH	--	--	--	--	--	--	--	--	--	--
						ML	--	--	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	8.00	92.00	10.00	90.00
						IN	--	--	--	--	--	--	--	--	--	--
						EA / Ceph	--	--	--	--	--	--	0.00	1.00		
						AA / YRI	--	--	--	--	--	--	--	--	--	--
				A	G		A	G	A	G	A	G	A	G	A	G
3	i1/-1459(G/A)	rs12720464	Intron 1	Interferon regulatory factor 2, / AREB6	GATA-binding factor 1, / Ecotropic viral integration site 1 encoded factor	CH	--	--	--	--	22.90	77.10	--	--	--	--
						ML	--	--	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	25.00	75.00	28.00	72.00
						IN	--	--	--	--	--	--	--	--	--	--
						EA / Ceph	--	--	--	--	0.00	1.00	0.00	1.00	--	--
						AA / YRI	--	--	--	--	0.00	1.00	--	--	--	--
				GA	Del GA		GA	Del GA	GA	Del GA	GA	Del GA	GA	Del GA	GA	Del GA
4	i1/-1423(DelGA)		Intron 1	--	--	CH	--	--	--	--	--	--	--	--	--	--
						ML	--	--	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	1.00	0.00	--	--
						IN	--	--	--	--	--	--	--	--	--	--
						EA / Ceph	--	--	--	--	--	--	99.50	0.50	--	--
						AA / YRI	--	--	--	--	--	--	--	--	--	--
				CCATC	Del CCATC		CCATC	Del CCATC	CCATC	Del CCATC	CCATC	Del CCATC	CCATC	Del CCATC	CCATC	Del CCATC
5	i1/-1132(DelCCATC)		Intron 1	Prostate-derived Ets factor	--	CH	--	--	--	--	--	--	--	--	--	--
						ML	--	--	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	96.30	3.70	--	--
						IN	--	--	--	--	--	--	--	--	--	--
						EA / Ceph	--	--	--	--	--	--	1.00	0.00	--	--
						AA / YRI	--	--	--	--	--	--	--	--	--	--
				T	C		C	T	C	T	C	T	C	T	C	T
6	i1/-1017(T/C)		Intron 1	--	Aryl hydrocarbon receptor, / Tax/CREB complex, / E2F	CH	--	--	--	--	--	--	--	--	--	--
						ML	--	--	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	8.00	92.00	10.00	90.00
						IN	--	--	--	--	--	--	--	--	--	--
						EA / Ceph	--	--	--	--	--	--	1.60	98.40	--	--
						AA / YRI	--	--	--	--	--	--	--	--	--	--
				T	C		C	T	C	T	C	T	C	T	C	T
7	i1 /-824 (T/C)		Intron 1	--	--	CH	--	--	--	--	--	--	--	--	--	--
						ML	--	--	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	0.50	99.50	--	--
						IN	--	--	--	--	--	--	--	--	--	--
						EA / Ceph	--	--	--	--	--	--	0.00	1.00	--	--
						AA / YRI	--	--	--	--	--	--	--	--	--	--
				A	G		A	G	A	G	A	G	A	G	A	G
8	i1/-755(A/G)		Intron 1	IRF-7, / CUT-homeodomain transcription factor Onecut-2, / Gfi-1B	CBFA1	CH	100	0	--	--	--	--	--	--	--	--
						ML	100	0	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	1.00	0.00	98.00	2.00
						IN	100	0	--	--	--	--	--	--	--	--
						EA / Ceph	100	0	--	--	--	--	99.50	0.05	--	--
						AA / YRI	100	0	--	--	--	--	--	--	--	--
				A	G		A	G	A	G	A	G	A	G	A	G
9	i1/-432(A/G)		Intron 1	NMP4, / E2F, / Nuclear factor of activated T-cells, / STAT6	FOXK2, / Ikaros 1, / RBP-Jkappa/CBF1,	CH	100	0	--	--	--	--	--	--	--	--
						ML	100	0	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	--	--	98.00	2.00
						IN	100	0	--	--	--	--	--	--	--	--
						EA / Ceph	100	0	--	--	--	--	--	--	--	--
						AA / YRI	100	0	--	--	--	--	--	--	--	--
				A	G		A	G	A	G	A	G	A	G	A	G
10	i1/-274(A/G)		Intron 1	--	Neuron-restrictive silencer factor, / X-box binding protein RFX1	CH	0.00	100.00	--	--	--	--	--	--	--	--
						ML	0.00	100.00	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	--	--	--	--
						IN	0.00	100.00	--	--	--	--	--	--	--	--
						EA / Ceph	0.00	100.00	--	--	--	--	--	--	--	--
						AA / YRI	1.12	98.88	--	--	--	--	--	--	--	--
				T	C		C	T	C	T	C	T	C	T	C	T
11	i1/-223(A/G)		Intron 1	Ecotropic viral integration site 1 encoded factor	--	CH	100.00	0.00	--	--	--	--	--	--	--	--
						ML	100.00	0.00	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	--	--	--	--
						IN	100.00	0.00	--	--	--	--	--	--	--	--
						EA / Ceph	99.47	0.53	--	--	--	--	--	--	--	--
						AA / YRI	100.00	0.00	--	--	--	--	--	--	--	--
				C	T		C	T	C	T	C	T	C	T	C	T
12	i1/-146(C/T)		Intron 1	--	Octamer-binding factor 1	CH	0.00	100.00	--	--	--	--	--	--	--	--
						ML	0.00	100.00	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	--	--	--	--
						IN	0.00	100.00	--	--	--	--	--	--	--	--
						EA / Ceph	0.00	100.00	--	--	--	--	--	--	--	--
						AA / YRI	0.56	99.44	--	--	--	--	--	--	--	--
				A	T		A	T	A	T	A	T	A	T	A	T
13	i1/-60(A/T)		Intron 1	Prostate-specific homeodomain protein NKX3.1	--	CH	100.00	0.00	--	--	--	--	--	--	--	--
						ML	100.00	0.00	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	--	--	--	--
						IN	100.00	0.00	--	--	--	--	--	--	--	--
						EA / Ceph	100.00	0.00	--	--	--	--	--	--	--	--
						AA / YRI	96.07	3.93	--	--	--	--	--	--	--	--
				A	G		A	G	A	G	A	G	A	G	A	G
14	i1/-41(A/G)	rs21886524	Intron 1	Nuclear factor Y	HEN 1, / Olfactory neuron-specific factor	CH	90.22	9.78	87.80	12.20	85.40	14.60	--	--	--	--
						ML	95.11	4.89	--	--	--	--	--	--	--	--
						JP	--	--	88.60	11.40	--	--	89.40	10.60	90.00	10.00
						IN	100.00	0.00	--	--	--	--	--	--	--	--
						EA / Ceph	99.50	0.50	100.00	0.00	97.90	2.10	1.00	0.00	--	--
						AA / YRI	99.50	0.50	100.00	0.00	100.00	0.00	--	--	--	--
				C	G		C	G	C	G	C	G	C	G	C	G
15	e1/-145(C/G)		Exon1	--	MEF3	CH	99.46	0.54	--	--	--	--	--	--	--	--
						ML	100.00	0.00	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	96.80	3.20	--	--
						IN	100.00	0.00	--	--	--	--	--	--	--	--
						EA / Ceph	100.00	0.00	--	--	--	--	100.00	0.00	--	--
						AA / YRI	100.00	0.00	--	--	--	--	--	--	--	--
				C	T		C	T	C	T	C	T	C	T	C	T
16	e1/-129(T/C)	rs3213619	Exon1	HMG box-containing protein 1	--	CH	4.35	95.65	7.80	92.20	6.20	93.80	--	--	--	--
						ML	4.89	95.11	--	--	--	--	--	--	--	--
						JP	--	--	6.80	93.20	--	--	8.00	92.00	10.00	90.00
						IN	1.05	98.95	--	--	--	--	--	--	--	--
						EA / Ceph	5.00	95.00	5.10	94.90	6.20	93.80	1.60	98.40	--	--
						AA / YRI	7.50	92.50	6.70	93.30	4.30	95.70	--	--	--	--
				A	G		A	G	A	G	A	G	A	G	A	G
17	e1/-43 (A/G)		Exon1	X-box-binding protein 1, / E2F, / MyT1	--	CH	100.00	0.00	--	--	--	--	--	--	--	--
						ML	100.00	0.00	--	--	--	--	--	--	--	--
						JP	--	--	--	--	--	--	--	--	--	--
						IN	100.00	0.00	--	--	--	--	--	--	--	--
						EA / Ceph	100.00	0.00	--	--	--	--	--	--	--	--
						AA / YRI	97.75	2.25	--	--	--	--	--	--	--	--

CH, Chinese; ML, Malay; JP, Japanese; IN, Indian; EA, European American; CEPH, European American from CEPH collection; AA, African American; YRI, Yoruba of African descent.

in a region of ~2 kb from e1/+28 to i1/-1707. The two Japanese groups examined four common SNPs [e1/-129(T/C), i1/-41(A/G), i1/-755(A/G), and i1/1017(T/C)], whereas our study only shares two common SNPs [e1/-129(T/C) and i1/-41(A/G)] with the Taniguchi et al. (2003) study and three common SNPs [e1/-129(T/C), e1/-145(C/G), and i1/-41(A/G)] with the Takane et al. (2004) study (Table 2). Taking into account these constraints, we proceeded to compare the results of the promoter-reporter activities from these three studies.

Although both Japanese groups generally observed less than 1.5-fold (<45%) differences in promoter activity for the different haplotypes they examined, we generally found differences of greater than 2-fold and as high as >6-fold (compare haplotype GCTAA**GC** and the most common haplotype, GCTAACT, in HeLa cells) in some haplotypes we studied in the two cell lines examined. The differences in the magnitude of changes in promoter activity of the different haplotypes between our results and the two Japanese groups could be attributed to the differences in the region of the MDR1 promoter and the polymorphisms or the cell lines examined. Indeed, we found that the same haplotype may have a different effect on different cell lines (Fig. 3C), possibly because of differences in the availability of specific regulatory transcription factors in the different cell lines. In KB3-1 cells, which do not support MDR1 expression, the MDR1 promoter activity is very low, and no differences were observed among the different haplotypes.

Taniguchi et al. (2003) found a statistically significant but small ~13% decrease in promoter activity of haplotype **C**G**CGC**, comprising the minor alleles of SNPs e1/-129(T/C) and i1/-41(A/G), compared with the most common TGTAT haplotype. Likewise, we observed that haplotype GCTA**GCC**, also containing the minor alleles of the same SNPs, resulted in ~52% decrease, albeit not statistically significant, in promoter activity in HeLa cells but no difference in HEK293 cells (Fig. 3C) compared with the most common GCTAACT haplotype. In contrast, results from Takane et al. (2004) showed that haplotypes 2 and 3, which carry the minor alleles of SNPs e1/-129(T/C) and i1/-41(A/G), resulted in a 40 and 32% increase in MDR1 promoter activity, although they did not examine whether these increases were statistically significant. The differences in the results are likely because of differences in the region of SNPs examined and the differences in the additional polymorphisms in the haplotype(s) examined by the two groups. When SNP e1/-145(C/G) was changed to the minor G allele, the MDR1 promoter activity was found to decrease by 28% in HepG2 cells (haplotype 6 in Takane et al., 2004) and by 76% in HeLa cells (Fig. 3C), but no differences were observed in HEK293 or KB3-1 cells. It is noteworthy that haplotype 9 in Takane et al. (2004), which contains the minor allele of SNP e1/-129(T/C), resulted in an ~30% increase in MDR1 promoter activity. We also observed a statistically significant 147% increase in MDR1 promoter activity in a haplotype in which only SNP e1/-129(T/C) was changed to its minor allele in HEK293 cells (Fig. 3C). However, this same haplotype resulted in 75% decrease in MDR1 promoter activity in HeLa cells highlighting the cell type specificity of polymorphisms at the MDR1 promoter region. Further experiments involving additional different cell types (preferably primary cells) and different tissue types will be necessary to verify the inference. If the inferences are true, it would highlight the dynamic nature of polymorphisms in which specific SNP haplotypes differentially alter function (e.g., promoter activity) in different cell types and implicate the complexities of the effect of polymorphisms on different cell/tissue types in our body.

In summary, this report highlights three interesting properties of polymorphisms at the *MDR1* promoter. First, only few and low-frequency (<10%) polymorphisms are observed at the *MDR1* promoter region, suggesting that this region may be quite invariant. Second, the distribution of polymorphisms that functionally affect MDR1 promoter activity is varied in the different populations with Chinese being more similar to the Malays and the European Americans being more similar to the African Americans. Third, polymorphisms affect the MDR1 promoter activity in a cell type-specific manner and the effect of two or more haplotypes on the MDR1 promoter activity is additive. Nonetheless, it may be necessary to examine the in vivo MDR1 expression of these promoter polymorphisms and to extend this study to include a comprehensive screening of a larger region upstream the TLSS in several different populations to identify additional SNPs and to evaluate the functional significance of the identified SNPs or haplotypes of SNPs in several different cell types or even under several different external stimuli.

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