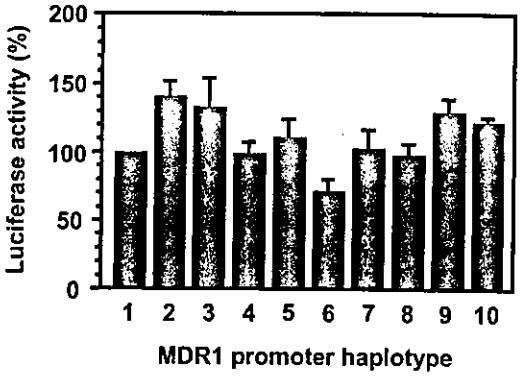

Fig. 3. *MDR1* promoter region reporter gene constructs and their relative luciferase activity levels. The value for the haplotype 1 construct was set at 100%. Each value is the mean ± S.D. of relative luciferase activity from four to five experiments.

rare, the C allele at position −129 may be associated with high level of expression of P-glycoprotein, resulting in an increase in transport activity and then a decrease in tacrolimus bioavailability after oral administration. In contrast to these haplotypes, haplotype 6 (G–1459aA and C–145G variant) was associated with a low level of transcriptional activity in hepatoma cells. We found that the G–1459aA variant inhibits the binding of unknown transcriptional factor to DNA. Therefore, allelic variants not only in the coding region but also in the promoter region of the *MDR1* gene, may be important to the interindividual difference in P-glycoprotein expression.

We determined the interindividual difference in methylation status in the proximal promoter region of the *MDR1* gene using seven placentas whose mRNA levels varied considerably. Extensive methylation of the *MDR1* gene assembled into chromatin enriched with methylated CpG binding protein interfered with the binding of transcription factors to their elements, resulting in transcriptional repression of the gene (Jin and Scotto, 1998; El-Osta et al., 2002). In this study, the region upstream of the promoter region was relatively well methylated, but individual differences in methylation status were unlikely to cause large variation in MDR1 mRNA expression. Nakayama et al. (1998) reported that methylation at CpG sites near the Y box was important for *MDR1* gene expression and was associated closely with clinical outcome in acute myeloid leukemia. However, we did not find any methylation around either the Y box or GC box element. Similar results for methylation status in the promoter region have been reported in CD8-positive cells (Fryxell et al., 1999). The element Sp1 protects the promoter from de novo methylation (Brandeis et al., 1994). Macleod et al. (1994, 1998) have suggested that the presence of a functional element within the GC-rich domain maintains the methylation-free status. Accordingly, although the proximal promoter of the *MDR1* gene is important for regulating basal gene expression in normal human tissues, epigenetic status

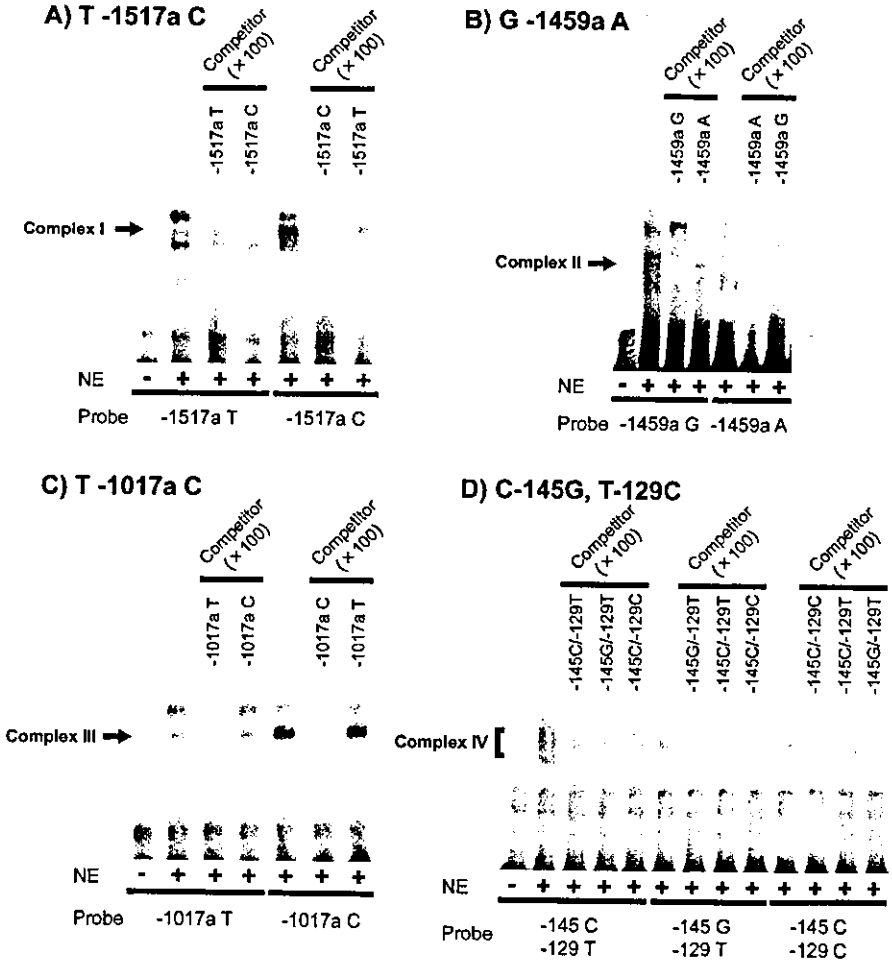

Fig. 4. EMSA analysis with oligonucleotides corresponding to variants (A, T-1517aC; B, G-1459aA; C, T-1017aT; and D, C-145G and T-129C) in the *MDR1* gene promoter. The nuclear extracts (NE; 5 μg of protein) from HepG2 cells were incubated with ^{32}P-labeled oligonucleotides. Specificity of nuclear protein binding was demonstrated by a 100-fold molar excess of unlabeled oligonucleotide. Data are representative of three similar experiments.

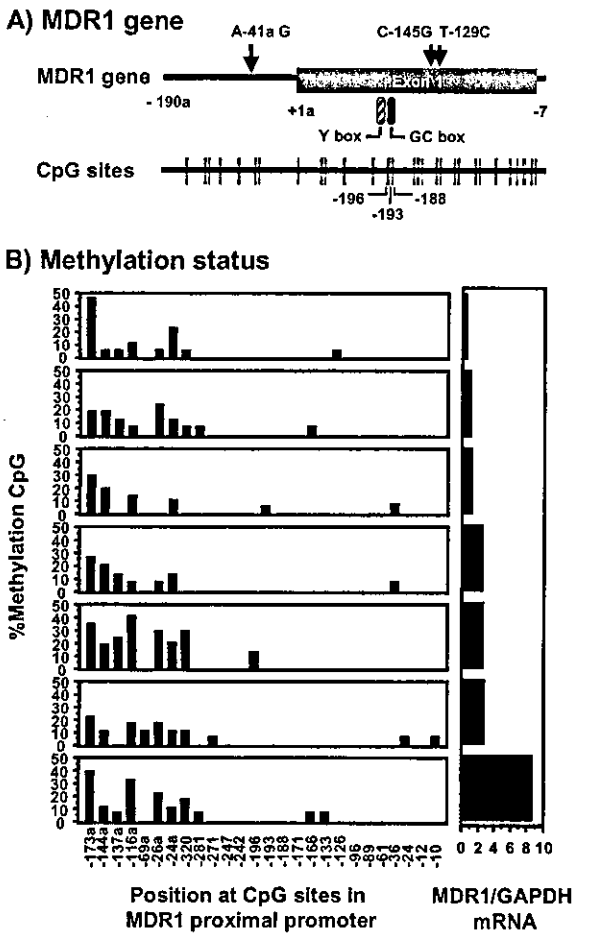

Fig. 5. A, location of CpG sites in the *MDR1* promoter region. The CpG sites are represented by short vertical bars. B, methylation status of individual sites of the *MDR1* promoter region and mRNA expression in placental tissues with the promoter haplotype 1/1 and 3435 C/C genotypes.

seems not to be associated with variability in the transcriptional activity of the *MDR1* gene.

In conclusion, we identified various variants in the promoter of the *MDR1* gene and investigated their effects on transcriptional activity and mRNA expression in the placenta. Among these variants, the promoter haplotypes containing T–1517aC, T–1017aC, and T–129C were particularly associated with high level of transcriptional activity and mRNA expression but independently of the coding SNP C3435T. Because these promoter haplotypes or SNPs are found at a relatively low frequency in Japanese and Caucasian populations, further study is needed to establish the impact of their allelic variants on drug disposition and responses in clinical situations. Interestingly, one study has shown a possibility of regulation by tissue-specific factors for the *MDR1* gene expression (Kohno et al., 1990). Also, Sundseth et al. (1997) have reported that an element just upstream of the Y box had opposite effects in different human carcinoma cell lines. Their results suggest that allelic variants in the promoter region of the *MDR1* gene contribute to the polymorphic expression of P-glycoprotein in a tissue-specific manner. Future studies may need to estimate the influence of *MDR1* promoter variants on transcriptional activity in various P-glycoprotein-expressing tissues.

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