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Comparative description of haplotype structure and genetic diversity of *MDR1* (*ABCB1*) in HIV-positive and HIV-negative populations

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Abstract

Human P-glycoprotein (P-gp), encoded by *MDR1* (*ABCB1*), is an efflux transporter with a wide specificity for substrates/drugs, including HIV protease inhibitors which are commonly used in HIV/AIDS treatment. Three single nucleotide polymorphisms (SNPs) in *MDR1* have been shown to affect P-gp expression and function, and may affect HIV/AIDS treatment outcome: 1236C>T [G412G, exon-12], 2677G>T/A [A893S/T, exon-21] and 3435C>T [I1145I, exon-26]. In the present study, our aims were (i) to compare the 3-SNP *MDR1* haplotype structure and genetic diversity between North American HIV-positive and HIV-negative individuals belonging to four major ethnic groups and (ii) to determine whether the haplotype structure and genetic diversity observed in these ethnically admixed populations differ from that in ethnically non-admixed populations. For these aims, we analyzed a cohort of 447 HIV/AIDS patients (White [*n* = 193], Black [*n* = 235], Hispanic [*n* = 17], and Asian [*n* = 2]). Results obtained for these patients were compared with the results for (i) HIV-negative individuals (*n* = 356) and (ii) various HapMap and Environmental Genome Project populations. We observed that the genetic characteristics of *MDR1* were largely consistent between HIV-positive and HIV-negative populations, but there were striking interethnic differences in the genetic characteristics of *MDR1* in both populations. Although it appeared that the genetic characteristics of *MDR1* were largely consistent between ethnically admixed and non-admixed populations, genetic characterization of the admixed populations remains to be done. Thus, our results provide useful comparative insights about the genetic characteristics of *MDR1* that could be extrapolated across population groups worldwide. For a meaningful interpretation of these results regarding HIV/AIDS treatment outcome, *MDR1* haplotype/diplotype structure data, genetic characterization of population admixture, and polymorphisms in other relevant drug transporter and/or metabolizing enzyme genes should be considered in future clinical studies.

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Keywords

MDR1; *ABCB1*; P-glycoprotein; HIV-positive; HIV-negative; Anti-HIV drugs; Protease inhibitors; North American ethnic groups; North American populations

1. Introduction

Highly active antiretroviral therapy (HAART), to treat HIV/AIDS, is a combination of at least three antiretroviral drugs from different classes: non-nucleoside reverse transcriptase inhibitor (NNRTI), nucleoside reverse transcriptase inhibitor (NRTI), and HIV protease inhibitor (PI) (Owen et al., 2006; Rodriguez-Novoa et al., 2006; Cressey and Lallemant, 2007; Telenti and Zanger, 2008). HAART has been shown to markedly decrease AIDS incidence and improve HIV/AIDS prognosis (Palella et al., 1998). However, viral replication in patients receiving HAART regimens can result in virologic rebound and emergence of drug-resistant strains, thus causing virologic failure and increasing the complexity of treatment (Saracino et al., 2008). A number of clinical factors, including adherence, regimen, and infection/disease stage at initiation of therapy, are well known to influence response to treatment with HAART (Conway, 2007; Easterbrook et al., 2008; Kitahata et al., 2009). Genetic variations in the absorption, metabolism, and disposition of antiretroviral drugs have also been implicated in differential therapeutic responses and toxicity patterns (Owen et al., 2006; Telenti and Zanger, 2008). However, the relationship of host genetic factors to the pharmacokinetics and pharmacodynamics of antiretroviral drugs in diverse populations across the globe is insufficiently understood. One such genetic factor is the multidrug resistance gene-1 (*MDR1* or *ABCB1*), which encodes a widely distributed membrane transport protein, P-glycoprotein (P-gp).

P-gp, an efflux transporter belonging to the ATP-binding cassette (ABC) protein superfamily, plays a significant role in the absorption, distribution, and elimination of a wide variety of substrates/drugs (Eichelbaum et al., 2004; Marzolini et al., 2004). Included in this list of substrates/drugs are many of the PIs: P-gp is known to transport the PIs amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir (Marzolini et al., 2004), and possibly atazanavir and lopinavir (Ma et al., 2007). Recently, a newer class of anti-HIV compounds, called integrase inhibitors, have been shown to be P-gp substrates (Cianfriglia et al., 2007). In addition to anti-HIV/AIDS drugs, P-gp is also involved in regulating the cellular entry of HIV-1 *in vitro* (Lee et al., 2000; Raviv et al., 2000).

The *MDR1* gene is localized to chromosome 7p21.1, spans a region of ∼200 kb, contains 28 (or 29) exons, and is highly polymorphic (Kroetz et al., 2003; Bodor et al., 2005). Of the more than 50 exonic single nucleotide polymorphisms (SNPs) in *MDR1* (Kroetz et al., 2003; Leschziner et al., 2006; Fung and Gottesman, 2009), SNPs 1236C>T [rs1128503] (exon-12, Gly412Gly), 2677G>T/A [rs2032582] (exon-21, Ala893Ser/Thr), and 3435C>T [rs1045642] (exon-26, Ile1145Ile) occur in different populations at high (>0.1) but various frequencies (Kroetz et al., 2003; Marzolini et al., 2004; Ozawa et al., 2004; Tang et al., 2004). These three SNPs are in strong but varied linkage disequilibrium (LD) in different populations, accounting for the two most common haplotypes (*MDR1*1*: 1236C-2677G-3435C, and *MDR1*13*: 1236T-2677T-3435T) (Tang et al., 2002; Kroetz et

al., 2003; Fung and Gottesman, 2009). Among these SNPs, 3435C>T has been the focus of attention in attempts to understand functional consequences of variation in *MDR1* (Morita et al., 2003; Owen et al., 2005b; Wang et al., 2005; Song et al., 2006; Kimchi-Sarfaty et al., 2007; Loeuillet et al., 2007; Fung and Gottesman, 2009). 3435C>T, in combinations with 1236C>T and/or 2677G>T, has been found by some, but not all, investigators to play a key role in modifying the function of MDR1. Although the mechanism of this effect is not fully understood at the molecular level, evidence suggests that 3435C>T affects the timing of P-gp co-translational folding, which results in changes in substrate specificity (Kimchi-Sarfaty et al., 2007; Fung and Gottesman, 2009).

In the present study, we analyzed blood samples from 447 North American HIV/AIDS patients and from 356 North American HIV-negative individuals for the 1236-2677-3435 haplotype structure and genetic diversity. We also obtained the 1236/2677/3435 SNP data for HapMap phase 1 and 2 and Environmental Genome Project populations from a publicly available database, the Genome Variation Server (G.V.S., http://gvs.gs.washington.edu/GVS/). The major aims of the present study were (i) to compare the *MDR1* haplotype structure and genetic diversity between HIV-positive and HIV-negative individuals representing the same geographic/genetic population structure and (ii) to determine whether the *MDR1* haplotype structure and genetic diversity observed in these ethnically admixed populations differ from that in ethnically non-admixed populations. We have two main reasons to pursue these aims: first, it has been shown that *MDR1* locus has recently undergone positive selection, most likely due to environmental factors, which may include xenobiotics and pathogens (Tang et al., 2004; Wang et al., 2007). Although, to our knowledge, no report has indicated genetic changes in *MDR1* associated with HIV, HIV-infected patients showed a significant increase in P-gp expression in $CD4^+$ and $CD8^+$ T cells (Andreana et al., 1996), in *MDR1* mRNA levels and P-gp expression in placentas (Camus et al., 2006), and in *MDR1* mRNA levels in peripheral blood mononuclear cells (PBMCs) (Turriziani et al., 2008). In this study, we sought to determine whether *MDR1* genetic characteristics are similar or different between HIV-positive and HIV-negative individuals.

The second reason to pursue these aims is that North American populations, belonging to four major ethnic groups, are known to be admixed (Parra, 2007; Kosoy et al., 2009). It has been shown that commonly used ethnic labels, such as self-identification and skin color, insufficiently and inaccurately represent pharmacogenetic diversity (Wilson et al., 2001), including *MDR1* genetic diversity (Leschziner et al., 2007; Estrela et al., 2008). In order to control for misrepresentation of individuals in such studies, either ancestry informative marker- or multilocus genotype-based clustering methods are generally used (Wilson et al., 2001; Estrela et al., 2008; Kosoy et al., 2009). Alternatively, by analyzing samples from populations of known ancestry, one can obtain a better understanding of comparative pharmacogenetic variation (Wang et al., 2007; Huang et al., 2008). Since ethnicity/race of individuals in both of our study groups, HIV-positive and HIV-negative, was self-identified, we performed genetic analyses of the 1236/2677/3435 SNP data from populations of known ancestry to compare our results.

2. Materials and methods

2.1. Subject samples and genomic DNA extraction

Packed blood pellets from HIV/AIDS patients (*n* = 447) were obtained in a coded manner from the Special Immunology Unit of the AIDS Clinical Trials Unit, Case Western Reserve University/University Hospitals of Cleveland, Cleveland, OH, where the patients were receiving HIV/AIDS treatment and care. All patients were adults. The mean (±SD) age at their first visit to the clinic was 35.3 ± 9 years, and 355 (79.4%) of them were males. The ethnic/racial distribution, based on self-identification, was as follows: White, *n* = 193 (43.2%); Black, *n* = 235 (52.6%); Hispanic, *n* = 17 (3.8%); and Asian, *n* = 2 (0.4%). Their average nadir $CD4^+$ T cell count was 128.2 ± 134.4 cells/mm^3, and their highest plasma HIV RNA level was 334,441 ± 745,526 copies/ml. All patients provided written informed consent for de-identified clinical data and specimen collection, storage, and usage in genetic and non-genetic studies. The data and specimen collection protocol was approved by the Institutional Review Board of University Hospitals of Cleveland.

Samples from healthy North American random blood donors (*n* = 356), whose ethnicity/race was self-identified at the time of sample collection, were obtained from the National Histocompatibility Laboratory, American Red Cross/University of Maryland Medical System, Baltimore, MD (Zimmerman et al., 1999). Among these individuals were Caucasian-Americans (C.A., *n* = 87), African-Americans (Af.A., *n* = 87), Asian/Oriental-Americans (A.O., *n* = 92), and Hispanic-Americans (H.A., *n* = 90). Blood samples from these donors were collected under protocols, including the procedures for informed consent, approved by the respective institutional review boards.

Ethnically, we used the terms White and Black for HIV/AIDS patients, and C.A. and Af.A. for HIV-negative individuals, as these terms were used in the patient-care database and in previous study (Zimmerman et al., 1999), respectively.

DNA was extracted from 200 μl of packed blood pellets from HIV/AIDS patients and whole-blood samples from random donors using the QIAamp 96 spin blood kit (QIAGEN, Valencia, CA), as previously described (Mehlotra et al., 2007b).

2.2. Polymerase chain reaction (PCR) and agarose gel electrophoresis

PCR primers were designed based on the *MDR1* sequence in GenBank (accession number AY910577) to selectively amplify *MDR1* exon-12 (1236C>T), exon-21 (2677G>T/A), and exon-26 (3435C>T) regions. Sequence homology and specificity of all primer sequences were checked using the BLASTn program (http://www.ncbi.nlm.nih.gov). The primer sequences, PCR buffer, amplification conditions, and method used to perform agarose gel electrophoresis are described in supplementary Table S1.

2.3. SNP genotyping

Genotyping of the *MDR1* SNPs was performed using a high-throughput, post-PCR oligonucleotide ligation detection reaction-fluorescent microsphere assay (LDR-FMA) on the *Bio-Plex* multiplex suspension array system (Bio-Rad Laboratories), as previously

described (Mehlotra et al., 2006, 2007b). The LDR primers were designed based on the *MDR1* sequence (GenBank accession number AY910577), and their sequences are provided in supplementary Table S2. The LDR conditions were 95 °C for 1 min, 95 °C for 15 s and 60 °C for 2 min (31 ×). The SNP genotypes were determined as previously described (Mehlotra et al., 2006, 2007b). The mean and 95% confidence interval (CI) of log-transformed fluorescence values corresponding to each genotype are presented in Table 1.

2.4. Genome Variation Server populations

We downloaded the 1236/2677/3435 SNP genotype data from G.V.S. for the following populations: Europeans from Utah, USA (CEU, $n = 113$), Africans from Ibadan, Nigeria (YRI, $n = 57$), Han Chinese from Beijing, China (HCB, $n = 81$), Japanese from Tokyo, Japan (JPT, $n = 81$) (all HapMap phase 1 and 2 populations), Africans from USA (AD-EGP, $n = 12$), Asians from China and Japan (ASN-EGP, $n = 22$), and Hispanics from USA (HISP-EGP, $n = 22$) (all Environmental Genome Project populations). Using these data, we performed the same genetic analyses as were performed on all other data.

2.5. Statistical analysis

MDR1 SNP-allele frequencies were calculated and the Hardy-Weinberg (H-W) exact test (estimation of *P* value by the Markov chain method) was performed for each group of individuals ("population") using the GenePop program (http://genepop.curtin.edu.au/). Differences in allele and haplotype frequencies between populations were measured using Fisher's exact test (http://www.langsrud.com/fisher.htm). Haplotype and diplotype (genotype) frequencies were estimated using the SHEsis (http://analysis.bio-x.cn/myAnalysis.php) and HAP (http://research.calit2.net/hap/) programs, respectively. In order to analyze LD between SNPs, we calculated Lewontin's D'; and correlation coefficient (r^2) values using the SHEsis program. Haplotype diversity (heterozygosity, H; mean ± SE) and between-population genetic differentiation (F_{st}) were computed using the Arlequin 3.1 package (http://cmpg.unibe.ch/software/arlequin3). Genetic diversity and differentiation values range from 0 to 1, with 0 meaning no diversity (i.e., all individuals have the same haplotype) and 1 meaning that every individual has a different haplotype. For all statistical analyses, a P value < 0.05 was considered to be significant.

3. Results

3.1. MDR1 SNP-allele frequencies

The observed frequencies of the *MDR1* SNPs (1236C>T [exon-12], 2677G>T/A [exon-21] and 3435C>T [exon-26]) in HIV/AIDS patients are presented in Table 2. The observed frequencies of these SNPs in HIV-negative North American and G.V.S. populations are presented in supplementary Table S3. 1236T-, 2677T-, and 3435T-allele frequencies were significantly higher in White patients than those in Black patients (Table 2). Due to the limited number of Hispanic patients ($n = 17$), we did not compare their SNP-allele frequencies with those of White ($n = 193$) or Black ($n = 235$) patients. The SNP genotypes of the two Asian patients were: 1236CT ($n = 1$), 1236TT ($n = 1$); 2677GT ($n = 2$); and 3435CT ($n = 2$). No further analysis of these results was performed.

Among HIV-negative North American and G.V.S. populations, the “African” group was distinctive in that it had the highest prevalence of 1236C, 2677G, and 3435C alleles (Table S3). In the “African” group, the 2677T/A allele was absent in YRI population (Table S3). In the “Asian” group, the 2677A allele was absent in HCB and JPT populations, whereas it was present in moderate to high frequencies in A.O. and ASN-EGP populations (Table S3). Overall, the SNP-allele frequency results for HIV-negative C.A. and Af.A. populations were similar to those obtained for White and Black patients (Table 2), as well as to those previously reported for comparable populations (Kim et al., 2001; Kroetz et al., 2003). The SNP-allele frequencies in HIV-negative A.O. and H.A. populations were similar to those in other Asian (Tang et al., 2002; Kroetz et al., 2003; Sai et al., 2003; Xu et al., 2008) and Mexican-American populations (Kroetz et al., 2003), respectively.

We calculated expected genotype numbers for each of the SNPs in HIV-positive, HIV-negative, and G.V.S. populations. The expected numbers did not differ significantly from the observed numbers (data not shown), with only one exception: The 3435TT genotype in HIV/AIDS Black patients deviated from the H–W equilibrium (observed number = 15, expected number = 8.62; $\chi^2 = 4.728$; $P = 0.029$). Intriguingly, although there were fewer observed ($n = 60$) than expected ($n = 72.77$) 3435CT genotypes, the difference did not reach significance ($\chi^2 = 2.24$, $P = 0.134$). Recently, the 3435C>T SNP in a Vietnamese population ($n = 78$) was found not to be in the H–W equilibrium (Veiga et al., 2009).

Finally, in order to confirm our SNP-allele results based on LDR-FMA, we sequenced a total of 34 PCR products for exon-12 ($n = 1$), exon-21 ($n = 12$), and exon-26 ($n = 21$) regions. We observed 100% concordance between the results from LDR-FMA and those from direct PCR sequencing.

3.2. Linkage disequilibrium between MDR1 SNPs

We performed LD analysis among *MDR1* SNP pairs by calculating both D' and r^2 values. The LD values in HIV/AIDS patients are presented in Table 3 and those in HIV-negative North American and G.V.S. populations are presented in supplementary Table S4. The North American populations are the result of very recent human migration and mixture. Although it might provide some valuable information (Cavaco et al., 2005; Mehlotra et al., 2007a), pharmacogenetic data from these populations should be evaluated with caution. The pattern and extent of LD (by D' values) were similar between White and Black patients; r^2 values for the 1236-2677 and 2677-3435 SNP pairs were noticeably lower in Black patients compared with those in White patients (Table 3). Similar results were obtained for HIV-negative C.A. and Af.A. populations (Table S4). At a glance, the pattern and extent of LD in Hispanic patients were similar to those in White and Black patients (Table 3), and also to those in the “Hispanic” group (Table S4). The subtle differences observed between Hispanic patients and the “Hispanic” group could be due to the fact that Hispanic populations within the continental US are heterogeneous in terms of culture, country of origin, and genetic composition (Dorado et al., 2007). Finally, in the “Asian” group, we noticed a much lower D' value for the 1236-2677 SNP pair in HCB and JPT populations than that in A.O. and ASN-EGP populations. This result was intriguing because the ASN-EGP population includes HCB ($n = 11$) and Japanese ($n = 11$) individuals (http://www.ncbi.nlm.nih.gov/

projects/SNP/snp_viewTable.cgi?pop_id=1475). Nevertheless, the overall pattern and extent of LD in HIV-negative A.O. population were similar to those in G.V.S.-Asian populations (Table S4).

Thus, our LD results did not indicate any considerable difference between HIV-positive and HIV-negative populations, or between admixed North American and non-admixed G.V.S. populations. These results are in agreement with those previously reported for comparable populations (Tang et al., 2002; Kroetz et al., 2003; Tang et al., 2004; Wang et al., 2007).

3.3. MDR1 3-SNP haplotype profiles, diversity, and between-population differentiation

We inferred *MDR1* 3-SNP haplotype profiles using the SHEsis program. The haplotype profiles in HIV/AIDS patients are presented in Table 4 and those in HIV-negative North American and G.V.S. populations are presented in supplementary Table S5. Assuming random association among the three *MDR1* SNPs, one can predict 12 different haplotypes (2 × 3 × 2). However, only four haplotypes would be possible if mutations yielding new alleles are the only evolutionary forces acting to create new haplotypes, and other forces, such as recombination, recurrent mutation, or gene conversion, do not occur (Bonnen et al., 2002). Grouped together, we inferred a total of 10 different haplotypes in White patients—"European" group, seven in Black patients—"African" group, and nine haplotypes each in the "Asian" group and in Hispanic patients—"Hispanic" group (Table 4 and Table S5). Of these haplotypes, only a few haplotypes together accounted for the majority of total chromosomes examined. We identified 5 such major haplotypes (C-G-C, C-T-C, C-G-T, T-G-C, and T-T-T) occurring at frequencies >0.1 in at least one population. The distribution of the two major haplotypes, C-G-C and T-T-T, differed significantly between White and Black patients (Table 4), and between HIV-negative C.A. and Af.A. populations (Table S5), which is a reflection of significant differences in SNP-allele frequencies between them (Table 2 and Table S3), and confirms previous observations (Kim et al., 2001; Kroetz et al., 2003).

Among HIV-negative North American and G.V.S. populations, we observed the highest prevalence of C-G-C haplotype in the "African" group (Table S5), as this group had the highest prevalence of 1236C, 2677G, and 3435C alleles (Table S3). In the "Asian" group, haplotypes T-T-T and T-G-C were observed at high prevalence. Haplotype C-A-C was present only in A.O. (prevalence 0.07) and ASN-EGP (prevalence 0.1) populations (Table S5), as the 2677A allele was absent in HCB and JPT populations (Table S3), whereas high prevalence of haplotype C-T-C was observed only in HCB and JPT populations. Haplotypes T-T-T, T-G-C, and C-A-C are predominant in many Asian populations (Tang et al., 2002; Xu et al., 2008). Finally, an overall comparison of the haplotype profiles indicated that the "Hispanic" group was more similar to the "European" group than to the other groups (Table S5).

Population-wise haplotype diversities are presented in Table 5. The levels of genetic diversity were high ($H \approx 0.6$ or higher) and similar in most of the populations. Lower haplotype diversities were observed for Black patients and Af.A., YRI, and AD-EGP populations. In this group, further reduced diversities were noticed for YRI and AD-EGP populations. One possible explanation for the observed lower haplotype diversities in all of

these populations could be significantly lower frequencies, or the absence of some, of the variant alleles (Table S3).

With *MDR1* genetic differences between White and Black patients, our next main question was; what is the level of genetic differentiation, based on 1236–2677–3435 haplotype, between these patient groups? As measured by F_{st}, we observed substantial differentiation between these patient groups (0.159). This level of differentiation was similar to that between CEU and YRI populations (0.193), considering that the 2677T/A allele is absent in YRI population. However, C.A. and Af.A. populations exhibited a much lower level of differentiation (0.064). In the continental US, the European ancestry contribution to African-American populations shows a substantial variation (Parra, 2007). Since our White and Black patients (the Northeast Ohio region), and C.A. and Af.A. populations (the Baltimore, MD, area) are from distinct areas, with a distance of roughly 400 miles, we analyzed levels of differentiation between White patients and C.A. population, and between Black patients and Af.A. population. We observed almost no differentiation between White patients and C.A. population (0.002), or between Black patients and Af.A. population (0.002). Therefore, the only plausible explanation for these results (F_{st} White–Black patients = 0.159, F_{st} C.A.–Af.A. populations = 0.064) is that the allelic/haplotypic differences seem to be more pronounced between White and Black patients than those between C.A. and Af.A. populations (Table 2/Table S3 and Table 4/Table S5).

3.4. MDR1 3-SNP diplotype configurations

Lastly, using the SNP genotype data, the HAP program was used to infer diplotype configurations in each population. The diplotype configurations in HIV/AIDS patients are presented in Table 6 and those in HIV-negative North American populations and G.V.S. populations are presented in supplementary Table S6. Mainly, there were substantial differences in the diplotype configurations between White and Black patients (Table 6); the same trend was noticed between C.A. and Af.A. populations (Table S6). Based on the diplotype configurations involving haplotypes T–G–C, C–T–C, and C–A–C, there was also a considerable difference between the “Asian” group and the remaining three groups (Table S6). Thus, it is important to determine whether these interethnic genetic differences in *MDR1*, together with other potentially significant polymorphisms in the *MDR1* coding, 1199G>A (Ser400Asn) (Woodahl et al., 2005), 571G>A (Gly191Arg) (Yang et al., 2008), and 3421T>A (Ser1141Thr) (Kroetz et al., 2003), as well as regulatory regions (Taniguchi et al., 2003; Loeuillet et al., 2007), are associated with different outcomes of protease inhibitor-based HIV/AIDS treatment.

4. Discussion

In the present study of the genetic characteristics of *MDR1*, utilizing samples from North American HIV-positive and HIV-negative populations as well as data from several populations included in human genetic variation projects, three major observations were made: (i) the genetic characteristics of *MDR1* are largely consistent between HIV-positive (White and Black patients) and HIV-negative (C.A. and Af.A.) populations, (ii) striking interethnic differences in the genetic characteristics of *MDR1* exist in both HIV-positive and HIV-negative populations, and (iii) the genetic characteristics of *MDR1* are largely

consistent between ethnically admixed and non-admixed (or in which admixture is supposedly low) populations. However, an important point to consider is that these conclusions are based on the most prevalent but only a limited number of SNPs. The most obvious and important questions are; what does this all mean in terms of HIV/AIDS treatment outcome, and where do we go from here?

4.1. Association between MDR1 SNPs/haplotypes and pharmacokinetics and/or efficacy of PIs and other classes of anti-HIV drugs

In the first study exploring association between *MDR1* SNPs and response to antiretroviral treatment (Fellay et al., 2002), HIV-infected Swiss patients were treated with nelfinavir- or efavirenz-containing regimens. Patients carrying the 3435TT genotype had significantly lower median concentrations of nelfinavir and efavirenz compared with those carrying the 3435CT or 3435CC genotype. The study also found that the 3435TT genotype was associated with significantly lower *MDR1* mRNA level and P-gp expression in PBMCs. Furthermore, in the treatment-naïve patients, the 3435TT genotype was associated with significantly higher mean $CD4^+$ counts six months after starting treatment, but had no effect on viremia decay (Fellay et al., 2002).

A number of subsequent studies have shown contrasting results: among HIV-infected North American children, treated with nelfinavir plus efavirenz-containing regimens, significantly higher 8-h postdose concentration and lower clearance rate of nelfinavir, and more rapid virologic response at week 8 were observed in children with 3435CT genotype than those in children with 3435CC or 3435TT genotype; no genotype had an effect on changes in $CD4^+$ lymphocyte counts (Saitoh et al., 2005). In antiretroviral-naïve HIV-infected North American patients, treated with nelfinavir- and/or efavirenz-containing regimens, 2677G>T and 3435C>T genotypes were not associated with plasma nelfinavir exposure or nelfinavir treatment failure (Haas et al., 2005). Among efavirenz recipients in the same study, the 3435TT genotype was not associated with plasma efavirenz exposure, but it was associated with increased virologic response and decreased emergence of efavirenz-resistant virus (Haas et al., 2005).

Clinical studies of *MDR1* haplotypes are limited in number and are also inconclusive. In HIV-infected patients, 2677G>T/A and 3435C>T genotypes, separately or as haplotypes, were not associated with clinical response to unboosted indinavir-containing regimens (Verstuyft et al., 2005). In healthy Canadian volunteers, 1236C>T, G2677G>T/A, and 3435C>T genotypes, separately or as haplotypes, were not associated with saquinavir pharmacokinetics, unboosted or boosted with ritonavir (la Porte et al., 2007). In contrast with these haplotype-based results, the 3435CT genotype was associated with virological efficacy in antiretroviral-naïve HIV-infected patients on an unboosted PI-containing regimen, but not in patients on a boosted PI-containing regimen (de la Tribonniere et al., 2008). In addition to these studies, 3435C>T genotypes, alone or in combination with 2677G>T genotypes, were not associated with progression to AIDS in HIV-infected patients (Bleiber et al., 2004; Hendrickson et al., 2008), but were significantly associated with a decreased risk of nevirapine-related hepatotoxicity (Haas et al., 2006; Ritchie et al., 2006).

In summary, substantial efforts have been made to decipher functional significance of *MDR1* polymorphisms in HIV/AIDS treatment. In many cases, the reported effects of *MDR1* polymorphisms have been inconsistent and, in some cases, contradictory. Therefore, the clinical relevance of *MDR1* polymorphisms in HIV/AIDS treatment remains questionable. This status strongly argues for identification of potential underlying causes of such discrepancies, as they might meaningfully determine association between *MDR1* genotype and HIV/AIDS treatment outcome.

4.2. Potential causes affecting MDR1 genotype–HIV/AIDS treatment outcome relationship

HIV infection/AIDS is intricate, and requires a complicated, multidrug treatment. Compared with the effects of *MDR1* polymorphisms on virologic/immunologic responses to anti-HIV drugs, only a limited number of clinical studies have focused on the effects of HIV/AIDS (Andreana et al., 1996; Meaden et al., 2001; Camus et al., 2006; Turriziani et al., 2008) and antiretroviral drugs (Lucia et al., 2002; Chandler et al., 2003; Ford et al., 2003) on *MDR1* mRNA levels, P-gp expression, and P-gp function. Although these reports are also confusing, it is becoming increasingly clear that HIV/AIDS treatment outcome may be influenced by the interrelationship between HIV and P-gp (Owen et al., 2005a). Our results, limited in their interpretation, did not indicate any difference in the genetic characteristics of *MDR1* between ethnically similar HIV-positive and HIV-negative populations.

Second, although there have been numerous studies on the 3435C>T polymorphism, not many have appeared regarding *MDR1* haplotypes. Using the common haplotype, 1236–2677–3435, may be a better way to interpret HIV/AIDS drug response and disease outcome measures than by studying only the 3435C>T polymorphism. Evidence suggests that each of the 1236C/T, 2677G/T/A, and 3435C/T alleles that make up the complete haplotype may contribute small but significant, additive or synergistic changes that lead to altered P-gp structure and function (Fung and Gottesman, 2009). In addition, our haplotype and diplotype data show substantial differences among ethnically diverse populations, which can be potentially relevant in interpreting the results of clinical studies conducted in various populations. Finally, a number of recently characterized *MDR1* SNPs certainly warrant examination. Among these are SNPs in the coding (Kroetz et al., 2003; Woodahl et al., 2005; Yang et al., 2008), non-coding (Colombo et al., 2005), and regulatory regions (Taniguchi et al., 2003; Loeuillet et al., 2007). Furthermore, what are the phenotypic effects of these SNPs when included with the common haplotype?

Third, it is important to genetically define populations, and detect and adjust for population stratification (Marzolini et al., 2004; Wang et al., 2005; Song et al., 2006; Estrela et al., 2008). It is well known that population stratification bias due to genetic admixture, as in North American populations, can confound the association of genetic factors with drug response. Genetic variants that influence response to a drug in one group/population might not have the same effect in another group/population due to differences in gene-gene and/or gene-environment interactions. Therefore, identification of admixture would be helpful. We attempted to address this issue, in a limited way, using two approaches: first, we compared our results for North American populations with the results for populations included in human genetic variation projects, and we found no considerable difference between the two

groups. Then, we performed genetic characterization of White and Black patients by using the Duffy blood group antigen (*FY*) as a population-specific marker. Among the three most common *FY* alleles, *FY*A, FY*B,* and *FY*B*^{null}, *FY*B*^{null} is a key marker for African ancestry (Parra et al., 1998; Zimmerman et al., 1999). Among the White patients, prevalence of the *FY*B*^{null} allele was 0.01, indicating the extent (1%) of the African ancestry contribution to European-American populations in the continental US (Parra et al., 1998). Among the Black patients, prevalence of the *FY*B*^{null} allele was 0.81 (*FY*A* [0.08], *FY*B* [0.11]), indicating the extent (19%) of the European ancestry contribution to African-American populations in the continental US (Parra et al., 1998). Thus, different fractions of ancestry from each ancestral sub-population in these patients need to be determined using other markers.

Lastly, it is likely that investigating polymorphisms in MDR1 alone is not sufficient in HIV/AIDS treatment outcome studies. High LD was found between *MDR1* 3435C>T and a synonymous coding SNP (rs1202283) in its adjacent polymorphic drug transporter gene, *ABCB4*; it was suggested that the coding sequence in one ABC transporter gene might act as a regulatory region for the expression of an adjacent ABC transporter gene (Leschziner et al., 2006). Interestingly, the *MDR1* 2677T (Lamba et al., 2006) and 3435T (Goto et al., 2002; Lamba et al., 2006) alleles were associated with enhanced hepatic and intestinal expression levels of the drug metabolizing enzyme gene, cytochrome *P450 3A4* (*CYP3A4*), located ∼120 kb away from the *MDR1* locus. CYP3A4 (and CYP3A5) are involved in the metabolism of PIs and NNRTIs (Owen et al., 2006; Rodriguez-Novoa et al., 2006; Cressey and Lallemant, 2007; Telenti and Zanger, 2008). Collectively, these studies suggest that the *MDR1* locus should not be viewed in isolation. Furthermore, the molecular mechanism of P-gp induction by known inducers of its expression may be mediated by the nuclear receptors, pregnane X receptor (PXR) (Owen et al., 2004) and constitutive androstane receptor (Burk et al., 2005). It is possible that polymorphism(s) in *PXR* could have an impact on the expression of its target genes and, consequently, drug clearance. Recently, a novel association between *PXR* 63396C>T polymorphism and unboosted atazanavir plasma concentrations has been reported, suggesting that PXR is important in the regulation of disposition of this drug (Siccardi et al., 2008). Finally, polymorphisms in other drug transporter as well as drug metabolizing enzyme genes can also influence HIV/AIDS treatment outcome. Other potentially relevant drug transporters and their substrates, used as part of HAART, are: multidrug resistance protein-1 (MRP1, PIs), MRP2 (PIs), MRP4 (NRTIs), MRP5 (NRTIs), and breast cancer resistance protein (NRTIs and NNRTIs) (Colombo et al., 2005; Owen et al., 2005a, 2006). It has been reported that HIV infection can increase the mRNA levels of *MRP1, MRP4,* and *MRP5* in monocyte-derived macrophages (Jorajuria et al., 2004) and PBMCs (Turriziani et al., 2008). Highly polymorphic CYP2B6 is involved in the metabolism of efavirenz and nevirapine. Among a number of its functionally relevant polymorphisms, 516G>T (Gln172His) and 983T>C (Ile328Thr) in particular, separately or combined, could significantly affect plasma levels of these drugs (Mehlotra et al., 2006, 2007a).

In conclusion, this study provides a comprehensive and comparative description of the most prevalent SNP-based genetic characteristics of *MDR1* in HIV-positive and HIV-negative

populations representing the same geographic/genetic structure. In addition, our results provide useful insights about the genetic characteristics of *MDR1* in admixed as well as non-admixed populations that could be extrapolated across population groups worldwide. In light of results of previous studies, the interpretation(s) of our findings regarding HIV/AIDS treatment outcome is not clear at this point. Nevertheless, considering (i) haplotype/diplotype structure data, (ii) genetic characterization of population admixture, and (iii) polymorphisms in other relevant drug transporter and/or metabolizing enzyme genes in future clinical studies is likely to enhance our understanding of genetic factors determining response to antiretroviral agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

MDR1 genotype-specific fluorescence values.

Polymorphism	Genotype[a]	N[b]	Mean[c]	95% CI[c]
1236C>T	CC	134	1.63	0.02
	CT	156	−0.08	0.00
	TT	66	−1.64	0.03
2677G>T/A	GG	168	1.21	0.02
	GT	131	−0.13	0.00
	TT	39	−1.67	0.03
	GA	10	0.85	0.04
	TA	6	−1.41	0.07
	AA	2	−0.47	.04
3435C>T	CC	140	0.65	0.01
	CT	163	0.13	0.01
	TT	53	−1.27	0.02

[a] Eight out of 18 2677A allele-containing samples were confirmed by direct PCR sequencing.

[b] *N*=356, total number of North American individuals (C.A., *n* = 87; Af.A., *n* = 87; A.O., *n* = 92; H.A., *n* = 90).

[c] Log-transformed fluorescence values.

Table 2

MDR1 SNP-allele frequencies in HIV/AIDS patients of different ethnicities.

Polymorphism	Frequency (allele)	White (*n*=193)	Black (*n*=235)	Hispanic (*n*=17)
1236C>T	*f*(C)	0.58	0.82	0.76
	f(T)	0.42[a]	0.18[a]	0.24
2677G>T/A	*f*(G)	0.55	0.9	0.68
	f(T)	0.43[a]	0.09[a]	0.21
	f(A)	0.02	0.01	0.12
3435C>T	*f*(C)	0.48	0.81	0.76
	f(T)	0.52[a]	0.19[a]	0.24

[a] *P* values <0.001 between White and Black patients.

Table 3

Linkage disequilibrium between *MDR1* SNPs in HIV/AIDS patients of different ethnicities.

SNP pair	LD	White (*n* = 193)	Black (*n* = 235)	Hispanic (*n* = 17)
1236–2677	D'	0.957	0.831	0.875
	r^2	0.844	0.351	0.468
1236–3435	D'	0.842	0.692	0.832
	r^2	0.477	0.439	0.692
2677–3435	D'	0.863	0.776	0.695
	r^2	0.533	0.284	0.376

Table 4

Frequencies of *MDR1* haplotypes in HIV/AIDS patients of different ethnicities.

Haplotype[a]	White (*n* = 193)	Black (*n* = 235)	Hispanic (*n* = 17)
C–G–C	0.43[b]	0.75[b]	0.65
C–T–C	0	0	0
C–A–C	0.01	0.01	0.08
C–G–T	0.11[c]	0.05[c]	0
C–T–T	0.02	0	0
C–A–T	0.01	0	0.03
T–G–C	0.01	0.04	0
T–T–C	0.02	0.01	0.03
T–A–C	0	0	0
T–G–T	0	0.05	0.03
T–T–T	0.39[d]	0.08[d]	0.17
T–A–T	0	0	0

[a] 1236–2677–3435, as inferred by the SHEsis program.

[b] $P<0.001$ between White and Black patients.

[c] $P=0.029$ between White and Black patients.

[d] $P<0.001$ between White and Black patients.

Table 5

MDR1 haplotype-based heterozygosities in various groups.[a]

Group	Population	*H* (mean ± SE)
HIV-positive	White	0.655 ± 0.014
	Black	0.417 ± 0.028
	Hispanic	0.574 ± 0.096
HIV-negative	C.A.	0.638 ± 0.025
	Af.A.	0.496 ± 0.043
	A.O.	0.782 ± 0.016
	H.A.	0.681 ± 0.023
G.V.S.	CEU	0.788 ± 0.013
	YRI	0.371 ± 0.053
	HCB	0.831 ± 0.011
	JPT	0.830 ± 0.013
	AD-EGP	0.239 ± 0.113
	ASN-EGP	0.753 ± 0.054
	HISP-EGP	0.691 ± 0.042

[a] *MDR1* haplotype 1236–2677–3435.

Table 6

Prevalence (number) of *MDR1* diplotypes in HIV/AIDS patients of different ethnicities.[a]

Diplotype	White (*n*=193)	Black (*n*=235)	Hispanic (*n*=17)
CGC–CGC	46	140	8
CGC–TTT	54	29	2
TTT–TTT	35	2	1
CGC–CAC	1	4	1
CGC–CGT	15	17	0
CGC–CTT	1	1	0
CGC–CAT	0	1	1
CGC–TGC	2	12	0
CGC–TTC	3	2	1
CGC–TGT	0	15	1
CAC–TTT	4	0	2
CGT–CGT	4	0	0
CGT–CTT	2	0	0
CTT–CTT	1	0	0
CAT–TTT	2	0	0
TGC–TGC	0	1	0
TGC–TTC	0	1	0
TGC–TGT	0	2	0
TGT–TGT	0	2	0
TGT–CGT	0	1	0
TTT–CTC	0	1	0
TTT–CGT	16	0	0
TTT–CTT	1	0	0
TTT–TGC	2	0	0
TTT–TTC	4	0	0
TTT–TGT	0	4	0

[a] Inferred by the HAP algorithm.