MDR1 Genetic Polymorphism Does Not Modify either Cell Permissiveness to HIV-1 or Disease Progression before Treatment

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Nonphysiological overexpression of the ABC transporter P-glycoprotein (P-gp), which is encoded by *MDR1*, has been associated with reduced susceptibility to human immunodeficiency virus (HIV) type 1 infection in vitro. We analyzed (1) the expression and genotype of *MDR1* and their relationship to HIV-1 permissiveness of CD4$^+$ T cells from 128 healthy blood donors and (2) the role that alleles of *MDR1* exons 21 and 26 play in modifying disease progression in 411 HIV-1–infected individuals. Differences in physiological levels of *MDR1* expression did not modify HIV-1 infection in vitro, nor did *MDR1* alleles and haplotypes significantly influence either permissiveness to infection in vitro or disease progression in vivo before the initiation of treatment.

The *MDR1* gene codes for the ABC transporter P-glycoprotein (P-gp). Protease inhibitors are substrates (as well as inhibitors and/or inducers) of this transporter. The identification of polymorphisms in the *MDR1* gene that are associated with changes in transport function spurred much research, including that in the field of HIV infection. The current state of knowledge has been reviewed recently by Kim [1]. In addition to the interest in the significance of P-gp in HIV therapeutics, there have been 3 reports on the potential role of P-gp in modulating cell susceptibility to HIV infection, independent of its role in drug transport [2–4]. These reports demonstrated a 50–70-fold decrease in viral production in cells overexpressing P-gp, effects that were partially reversed by selective inhibition of the transporter. The proposed mechanism includes the association between the transporter and glycolipid-enriched membrane domains (lipid rafts) [5], important sites for viral fusion and assembly and, thus, the disruption of HIV binding to the cell.

The experiments in the 3 reports cited were conducted in vitro and used nonphysiological levels of P-gp expression in cell lines. The reports pointed out that the possible role that P-gp plays in modifying the natural evolution of disease in vivo in patients not receiving antiretroviral therapy should be assessed. We addressed this issue by investigating (1) whether variation in *MDR1* expression of purified CD4$^+$ T cells would correlate with differences in permissiveness to HIV infection under standardized infection conditions in vitro and (2) whether *MDR1* polymorphisms associated with differences in *MDR1*/P-gp expression would influence the progression of HIV infection in vivo before the initiation of antiretroviral treatment.

Subjects, materials, and methods. Four hundred eleven participants were recruited from within the genetics project of the Swiss HIV Cohort Study (http://www.shcs.ch). The ethics committees of all participant centers approved the study. Patients gave written, informed consent for genetic testing.

We established a collection of purified CD4$^+$ T cells from 128 white, healthy blood donors, and the T cells were isolated from peripheral blood mononuclear cells (PBMCs) by use of anti-CD4$^+$ magnetic beads (Miltenyi Biotech). Cells were cultured in RPMI 1640 (GIBCO-Invitrogen) with 20% fetal calf serum and 20 U of human IL-2/mL, at 37°C and in 5% CO_2. The cells were characterized with respect to their permissiveness to HIV-1 infection in vitro under standardized conditions. CD4$^+$ T cells (1.5×10^5) were infected with the R5 strain NL4-3BaL *env* (500 pg of p24). Both 5 and 7 days after infection, p24 was measured in the culture supernatant by ELISA (Abbott). Stocks of DNA and RNA were prepared to allow for genetic testing.

Cells from donors and DNA from patients were genotyped at *MDR1* exons 21 (G2677T) and 26 (C3435T) by *Taq*Man allelic-discrimination techniques (Applied Biosystems). For exon 21, the primers were 5′-GGACAAGCACTGAAAGATAAGAAAGA-3′ (forward) and 5′-TGAGGAATGGTTATAAACACATTCTT-AGA-3′ (reverse), and the probes were 5′-ACCTTCCCAGcAC-CTT-3′ (FAM) and 5′-CTTCCCAGaACCTT-3′ (VIC). For exon 26, the primers were 5′-TGCTGAGAACATTGCCTATGGA-3′ (forward) and 5′-GGCATGTATGTTGGCCTCCTT-3′ (reverse),

Received 16 June 2003; accepted 26 August 2003; electronically published 29 January 2004.
Financial support: Swiss National Science Foundation (grant 3345-100935); Swiss HIV Cohort Study (Swiss National Science Foundation, grant 3345-062041); Santos Suarez Foundation.

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The Journal of Infectious Diseases 2004; 189:583–6

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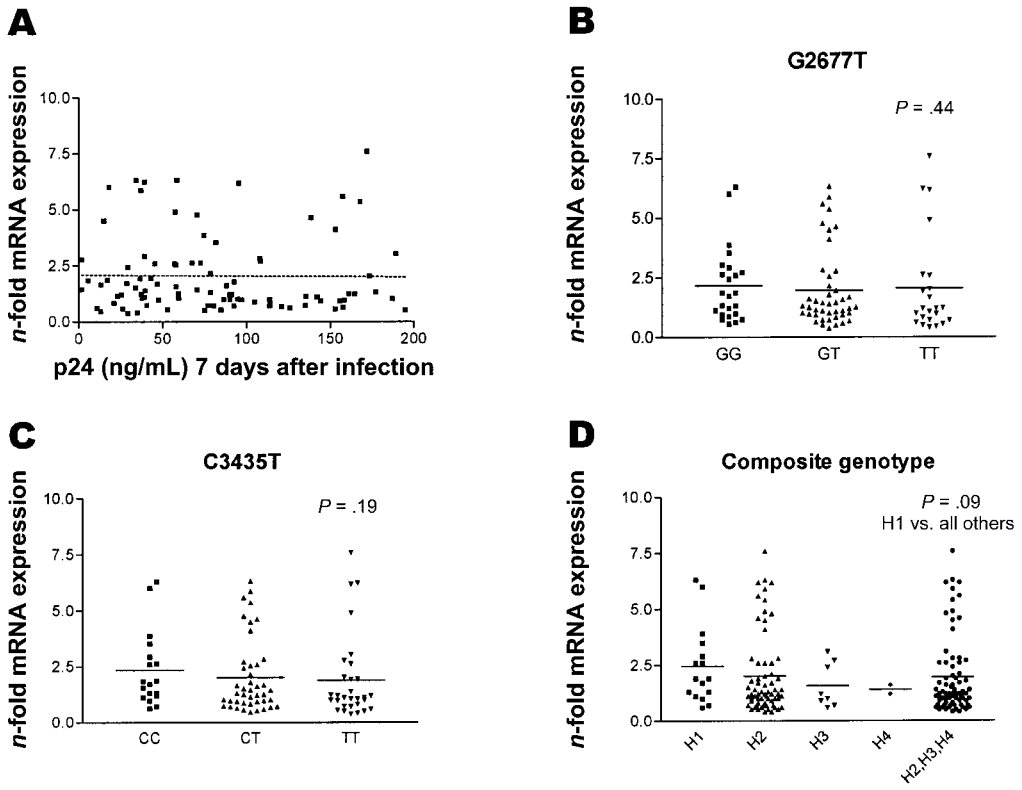

Figure 1. *MDR1* expression in CD4+ T cells, permissiveness to HIV-1 infection, and *MDR1* alleles. No association was observed between *MDR1* expression and permissiveness in CD4+ T cells from healthy blood donors *(A).* For the various alleles and haplotypes *(B–D),* an ordered trend (CC > CT > TT) was observed only for an association between the exon 26 genotypes at position 3435 and mRNA expression *(C).* The polymorphisms at *MDR1* exons 21 (G2677T) and 26 (C3435T) were analyzed both separately and as composite genotypes: H1 = 2677GG and 3435CC (wild type); H2 = 2677GT or TT and 3435CT or TT (2677T/3435T haplotype carrier); H3 = 2677GG and 3435CT or TT; and H4 = 2677GT or TT and 3435CC. The dotted line represents the regression line; solid lines represent the mean.

and the probes were 5′-AAGAGATcGTGAGGGC-3′ (FAM) and 5′-AAGAGATtGTGAGGGCA-3′ (VIC). Data were also analyzed with respect to the presence of the *MDR1*2* haplotype (2677T/3435T). Total RNA was extracted by use of an RNeasy MiniKit (Qiagen), from viable PBMCs conserved in liquid nitrogen, and was quantified by measurement of its optical density. The quantification of *MDR1* transcripts was performed by real-time polymerase chain reaction with primers designed to avoid amplification of genomic DNA; the primers were 5′-TGCTGAGAACATTGCCTATGGA-3′ (forward) and 5′-CCTTTGTCTCCTACTTTAGTGCTATATTTATTAG-3′ (reverse), and the probe was 5′-CCTGTGACACCACCCGGCTGTTGT-3′ (VIC/TAMRA). Results for the target gene were normalized to β-actin RNA levels by use of primers 5′-GATGACCCAGATCATGTTTGAGA-3′ (forward) and 5′-CACCGGAGTCCATCACGAT-3′ (reverse) and probe 5′-CCTGTACGCCTCTGGCCGTACCAC-3′ (VIC/TAMRA). Transcript levels were expressed in arbitrary units, calculated by dividing the normalized amplification threshold cycle by the score of a predefined sample.

Patients in whom ⩾2 measurements of CD4+ T cells were made before exposure to antiretroviral drugs and who were successfully genotyped for *MDR1* exons 21 (G2677T) and 26 (C3435T) were included in the analysis of in vivo progression of HIV. The trajectory of CD4+ T cell depletion in each patient was based on a repeat-measures random-effects model, by use of MLwiN software. Log_{10} CD4+ was modeled as a linear function of time and polymorphism group, with additional terms for sex, age range (16–29 years, 30–39 years, 40–49 years, or ⩾50 years), ethnicity (white or other), and risk group (injection drug use or non–injection drug use). Both the intercept and gradient of the trajectory of CD4+ T cells were allowed to vary between patients. The average time for CD4+ T cells to decline from 500 to 200 cells/μL was estimated for each polymorphism group. The polymorphisms of *MDR1* exons 21 (G2677T) and 26 (C3435T) were analyzed both separately and in combination as composite genotypes: H1 = 2677GG and 3435CC (wild type); H2 = 2677GT or TT and 3435CT or TT (2677T/3435T haplotype carrier); H3 = 2677GG and 3435CT or TT; and H4 = 2677GT or TT 3435CC.

Results. The quantification of *MDR1* transcripts in CD4+ T cells of healthy blood donors revealed that the mean ± SD interindividual variation in *MDR1* expression was 2.04 ± 1.73.

No correlation between *MDR1* expression and permissiveness to HIV-1 infection was observed (figure 1*A*). To determine whether polymorphisms in *MDR1* are associated with variation in *MDR1* expression in $CD4^+$ T cells, genotyping was performed at exon 21 (G2677T) and exon 26 (C3435T). The mean ± SD *n*-fold *MDR1* expression was 2.17 ± 1.56 in cells with 2677GG, 1.97 ± 1.64 in cells with 2677GT, and 2.05 ± 2.10 in cells with 2677TT ($P = .44$; figure 1*B*). An ordered trend (CC > CT > TT) was observed for an association between the exon 26 genotypes at position 3435 and mRNA expression—2.34 ± 1.67 for 3435CC, 2.03 ± 1.66 for 3435CT, and 1.89 ± 1.89 for 3435TT ($P = .19$; figure 1*C*)—and for H1 composite genotypes versus non-H1 composite genotypes ($P = .09$; figure 1*D*). Analysis of strict haplotypes (i.e., GG/CC vs. TT/TT vs. GG/TT vs. TT/CC) did not alter the results.

Specific genotypes did not lead to measurable differences in cell permissiveness to HIV infection in the in vitro system (figure 2*A*). Mean ± SD p24 values (ng/mL) 7 days after infection were 97.2 ± 69.7 for 2677GG, 92.0 ± 60.4 for 2677GT, and 87.1 ± 54.2 for 2677TT ($P = .92$); the corresponding figures were 93.1 ± 73.8 for 3435CC, 89.0 ± 56.1 for 3435CT, and 94.5 ± 60.8 for 3435TT ($P = .89$). The results were similar after adjustment for CCR5Δ32, CCR5 promoter polymorphism G59029A, and CCR2 64I in multivariate analysis (data not shown). The power was sufficient to detect 2-fold differences, in in vitro p24 levels, between the opposing homozygous groups (i.e., 2677GG vs. TT and 3435CC vs. TT); a sample size of ⩾1000 blood donors would have been necessary to statistically corroborate a 10% difference between these groups.

The influence of *MDR1* allelic variants on disease progression in vivo was analyzed by genotyping HIV-1–infected patients unexposed to potent antiretroviral therapy. For the 411 patients included in the analysis, the distribution of the *MDR1* exon 21 (G2677T) polymorphism was 35% GG, 46% GT, and 19% TT; for the *MDR1* exon 26 (C3435T) polymorphism, the distribution was 24% CC, 49% CT, and 27% TT. The average time for $CD4^+$ T cells to decline from 500 to 200 cells/μL was 3.68 years (95% confidence interval [CI], 3.09–4.56). For each genotype and haplotype, the estimated time and 95% CIs for the decline in $CD4^+$ T cells is shown in figure 2*B*. At *MDR1* exon 21, GG homozygocity was associated with a slightly steeper decline in $CD4^+$ T cells (3.5 years [95% CI, 2.85–4.56]), compared with TT (3.76 years [95% CI, 2.90–5.33]), whereas, at *MDR1* exon 26, CC was associated with a somewhat less-steep decline (3.79

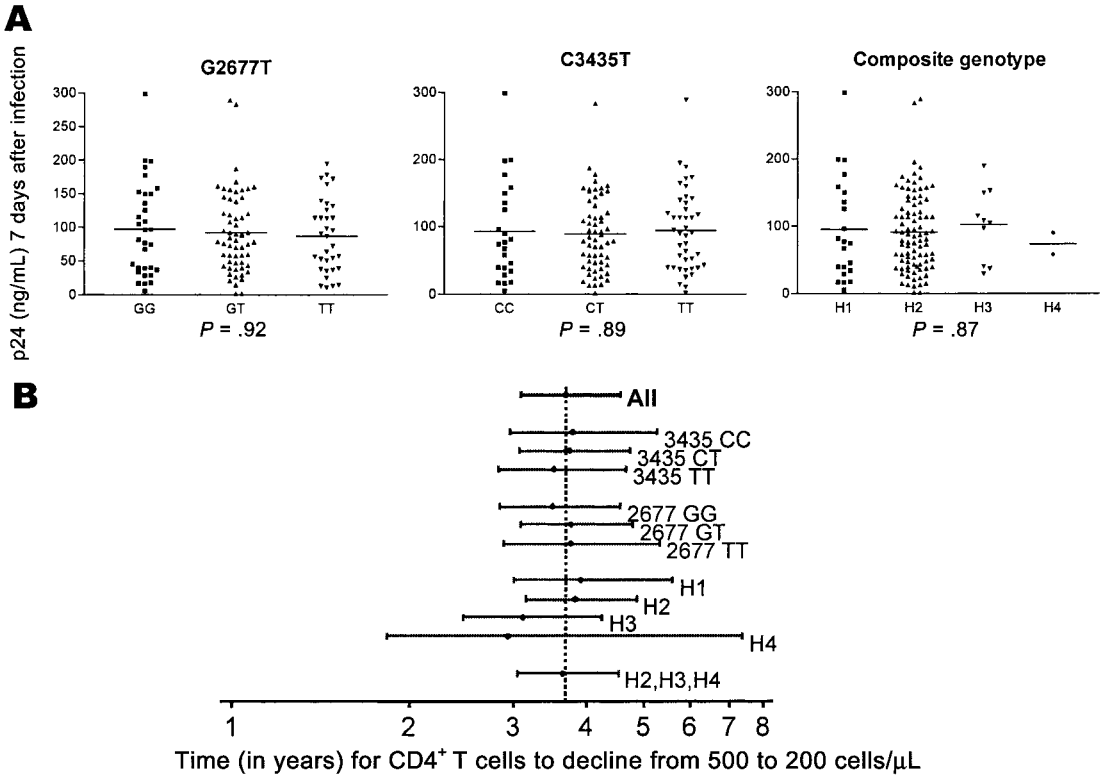

Figure 2. *MDR1* allelic variants and HIV-1 infection. *A,* Influence on $CD4^+$ T cells' permissiveness to HIV-1 infection in vitro. Cells of 128 healthy blood donors are analyzed. *B,* Influence of *MDR1* allelic variants on disease progression in vivo. The dotted line represents the average time necessary for $CD4^+$ T cells to decline. The trajectory of $CD4^+$ T cells in 411 HIV-1–infected patients was based on a repeat-measures random-effects model adjusted for sex, age group, ethnicity, and risk group. The polymorphisms at *MDR1* exons 21 and 26 were analyzed separately and as composite genotypes, H1 to H4. Lines represent the mean.

years [95% CI, 2.96–5.28]) compared with TT (3.52 years [95% CI, 2.83–4.66]). Analysis of composite genotypes showed a trend toward faster decline in $CD4^+$ T cells from H1 to H4 (H1 at 3.91 years [95% CI, 3.00–5.60] compared with H4 at 2.94 years [95% CI, 1.83–7.36]); however, none of these differences reached conventional levels of statistical significance.

Discussion. We observed 2- to 3-fold differences in *MDR1* expression in $CD4^+$ T cells from different individuals. This variation in physiological levels of *MDR1* expression did not appear to contribute to $CD4^+$ T cells' susceptibility to HIV-1 infection in vitro. However, the results do not negate the reports, by 3 independent research groups [2–4], of the effect of P-gp overexpression in vitro in cell lines, and they do not exclude effects that would have been uncovered by specific analysis of P-gp expression in vivo. Cells overexpressing P-gp, such as the L-MDR1 cell line [6], have an estimated 1000-fold-greater level of *MDR1* transcripts than do PBMCs.

MDR1 mRNA expression in purified $CD4^+$ T cells correlated with exon *MDR1* polymorphisms in the present study—although differences were not statistically significant—and with the expression of both *MDR1* and surface P-gp in PBMCs from HIV-1–infected individuals that has been reported in our previous study [7]. Although there is controversy regarding the biological relevance of *MDR1* polymorphisms, most published reports agree that there is an association between specific genotypes or haplotypes and the above-mentioned observed differences in either *MDR1* expression or P-gp function [1]. The reasons for this controversy are multiple, including that published reports on genotype/phenotype relationships have used various cell types (6 different cell sources), various ethnic origins for study participants (3 ethnic groups), and different probe (i.e., test) drugs (digoxin, fexofenadine, cyclosporine, tacrolimus, nelfinavir, rhodamine 123, and nortriptyline) [8] in analysis. In the present study, we used *MDR1* allelic variation as a surrogate for *MDR1* expression in vivo, and we assumed that transcript levels reflect protein expression at the membrane; this was because in our previous study we had shown, for the same set of samples, a good correlation between transcript and protein expression [7].

Ifergan et al. investigated, in 137 white individuals heavily exposed to HIV-1, whether *MDR1* variants are associated with risk of infection and concluded that the various alleles had no relevant influence [9]. Our present work suggests that the physiological differences in *MDR1* expression do not appear to have an influence on HIV-1 infection in the in vitro system used and that *MDR1* genotypes do not appear to modify disease progression in vivo in a cohort of HIV-1–infected individuals. Inclusion in the composite genotype/haplotype analysis of the linked allele in exon 12 (C1236T) should not alter our overall results and conclusion; however, we acknowledge that in vivo the 95% CIs of the estimated time for $CD4^+$ counts to decline are wide and that therefore our study cannot, with certainty, exclude a clinically relevant but small influence of *MDR1*/P-gp expression and polymorphisms on HIV-1 disease evolution before the initiation of treatment.

Swiss HIV Cohort Study Members. The members of the Swiss HIV Cohort Study are M. Battegay (chairman of the Scientific Board), M.-C. Bernard, E. Bernasconi, H. Bucher, Ph. Bürgisser, M. Egger, P. Erb, W. Fierz, M. Flepp (chairman of the Clinical and Laboratory Committee), P. Francioli (president of the SHCS, Centre Hospitalier Universitaire Vaudois, Lausanne), H. J. Furrer, M. Gorgievski, H. Günthard, P. Grob, B. Hirschel, C. Kind, Th. Klimkait, B. Ledergerber, U. Lauper, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, J.-C. Piffaretti, M. Rickenbach (head of the Data Center), C. Rudin (chairman of the Mother and Child Substudy), J. Schupbach, A. Telenti, P. Vernazza, Th. Wagels, and R. Weber.

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