ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2009, p. 1670–1672 Vol. 53, No. 4
0066-4804/09/$08.00+0 doi:10.1128/AAC.01494-08


# Detection of Human Immunodeficiency Virus (HIV) Type 1 M184V and K103N Minority Variants in Patients with Primary HIV Infection▽

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Received 7 November 2008/Returned for modification 19 December 2008/Accepted 16 January 2009

**We used an allele-specific real-time PCR assay to explore the presence of K103N and M184V minority species among primary human immunodeficiency virus (HIV) infections and their potential influence in HIV transmission. Thirty randomly chosen antiretroviral drug-naive patients lacking both the K103N and the M184V mutations as determined by conventional sequencing methods were studied, and K103N and M184V viral minority species were found in three (10%) and four (11%) patients, respectively.**

The activity of antiretroviral drugs (ARVs) can be impeded by the selection and transmission of drug resistance-associated mutations (4, 6, 11). Yet, few studies of the potential impact of such mutations on viral transmissibility have been carried out (13). Our group showed that mutations associated with protease inhibitors (PIs), thymidine analogues (thymidine-associated mutations), or nonnucleoside reverse transcriptase inhibitors (NNRTIs) were present in approximately 10 to 20% of newly infected patients but that the M184V mutation in reverse transcriptase (RT), associated with resistance to lamivudine and emtricitabine, was identified in only about 4% of such subjects (12). Since M184V is known to adversely affect viral replicative capacity as well as efficiency of RT initiation and function (3, 14), we speculated that this mutation might also affect human immunodeficiency virus (HIV) transmissibility.

The above-mentioned results were obtained using conventional sequencing methods that cannot detect minority variants that are present below a detection threshold of 20% of the total viral population. Therefore, it remained possible that viruses containing M184V might be transmitted as efficiently as other viruses but that the M184V mutation might revert to the wild type or be deselected in newly infected patients not receiving ARVs. As a consequence, M184V-containing viruses might be out-competed by wild-type viruses with higher replication capacity. To try to resolve this issue, we employed an allele-specific PCR assay (AS-PCR) to specifically detect the presence of minority species within the viral populations of newly infected individuals in the Montreal primary-HIV-infection (PHI) cohort.

**Study population.** A random sampling of 30 untreated patients enrolled between 2005 and 2007 in the Montreal PHI cohort were included and had been infected for less than 6 months, as described previously (12); none of these individuals possessed either the K103N or the M184V resistance mutation, as determined by bulk sequencing. All patients provided informed consent. Plasma HIV-1 RNA was measured using the Quantiplex HIV-1 RNA and bDNA systems (threshold, 50 copies/ml; Bayer Diagnostics).

**Drug resistance genotyping.** Viral RNA was extracted from plasma by using a QIAamp viral extraction kit (Qiagen, Mississauga, Ontario, Canada). Genotyping was performed by sequencing a 1,497-bp fragment of the HIV *pol* region (positions 2253 to 3749) spanning the entire protease (PR) and most of the RT region (codons 1 to 400), using Virco primers (Virco, BVBA, Mechelen, Belgium) with a BigDye Terminator cycle sequencing kit (version 1.1; Applied Biosystems, Foster City, CA) and an automated sequencer (ABI Prism 3130 genetic analyzer; Applied Biosystems).

**Quantification of minority resistance species by AS-PCR.** Plasma viral samples obtained during PHI that lacked either the M184V or the K103N mutation were evaluated by AS-PCR to detect viral populations that possibly carried these mutations (sensitivity, ≈1%); sensitivity and specificity were monitored using positive and negative controls. Purified PCR products of the genotyped samples were used as described previously (7, 10) in an assay in which the 5′ ends of forward primers were subjected to an inosine modification. The primers used were IN_K103N (5′-CCGCAGGGTTAAAAAAGAIC-3′; nucleotides [nt] 2839 to 2858) and Pol 3002 (5′-CTGTGGAAGCACATTGTACTG-3′; nt 2982 to 3002) for detection of K103N and IN_M184V (5′-CCAGACATAGTTATCTATCAATAIG-3′; nt 3075 to 3099) and N35 (5′-CCTACTAACTTCTGTATGTCATTGACAGTCCAGCT-3′; nt 3300 to 3333) for detection of M184V. Total viral populations were also amplified with primers Pol 2801 (5′-TCAAGACTTCTGGGAAGTTCA-3′; nt 2801 to 2821) and Pol 3122 (5′-TGCTGCCCTATTTCTAAGTCA-3′; nt 3122 to 3134), spanning RT amino acids 103 to 184. Results were expressed as proportions of mutant viruses in the total population. Real-time PCR was performed using a Rotor-Gene 6000 apparatus (Corbett Life Sciences), with a third-generation dsDNA intercalating dye termed SYTO9 (Invitrogen). DNA standards for quantification were prepared by PCR amplification. Specific K103N and M184V mutations were introduced by site-directed mutagen-

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▽ Published ahead of print on 26 January 2009.

TABLE 1. Viral drug resistance mutational profiles[a]

| Sample no. | Mutation(s) conferring resistance to: | | AS-PCR result | | |
|---|---|---|---|---|---|
| | Protease | RT | Plasma HIV-1 RNA level ($log_{10}$) | M184V | K103N |
| 1 | M36I, L63P/L | V108I/V, V179D, Y181Y/C | 4.6 | + | − |
| 2 | D30N, L33F, L63P, A71V, N88D | M41L, L210W, T215C | | | |
| 3 | L63P | L210F | | | |
| 4 | L10I, L63Q, V77I, I93L | None | | | |
| 5 | L63P | G190A | | | |
| 6 | V77I, D60K | None | | | |
| 7 | L63P, V77I, I93L | L210F | | | |
| 8 | L63A | None | | | |
| 9 | L63P | None | | | |
| 10 | L63L, V77I, I93L | L210F | 3.8 | + | − |
| 11 | L10I | M41L, E44D, L210W, T215D | 3.8 | + | − |
| 12 | M36V, L63P | None | | | |
| 13 | L63P, V77I, I93L | None | | | |
| 14 | L63P, V77I, I93L | V118I | | | |
| 15 | L63P, I93L | G333E | | | |
| 16 | L63P, V77I | G190A | | | |
| 17 | A71T, L63P, V77I, I93L | None | | | |
| 18 | L63P, K70R | None | 4.3 | − | + |
| 19 | L63P, V77I | None | | | |
| 20 | L63P | None | | | |
| 21 | L63P, A71T, V77I | None | 3.8 | − | + |
| 22 | L63P, V77I | A98S | | | |
| 23 | L63P, V77I | V118I | | | |
| 24 | L10I, M36I | V118I, L210 M | | | |
| 25 | L33V, L63P | L210F, G333E | 4.9 | + | − |
| 26 | L63P | None | | | |
| 27 | L63P, V77I | None | | | |
| 28 | L10I, L63P | None | | | |
| 29 | L63P, K70R | None | 4.8 | − | + |
| 30 | L63P, V77I | None | | | |

[a] All viral samples evaluated were subtype B.

esis (SDM) into HIV-1 subtype B pNL4-3 wild-type plasmid using a QuikChange SDM kit as specified by the manufacturer (Stratagene). For SDM of K103N, primers K103N B Fw (5′-CTG CAG GGT TAA AAC AGA ACA AAT CAG TAA CAG TAC TG-3′; nt 2839 to 2876) and K103N B Rev (5′-CAG TAC TGT TAC TGA TTT GTT CTG TTT TAA CCC TGC AG-3′; nt 2839 to 2876) were used. For M184V, primers M184V B Fw (5′-GAC ATA GTC ATC TAT CAA TAC GTG GAT GAT TTG TAT GTA GGA TC-3′; nt 3078 to 3121) and M184V B Rev (5′ GAT CCT ACA TAC AAA TCA TCC ACG TAT TGA TAG ATG ACTA TGT C-3′; nt 3078 to 3121) were employed.

**Detection of minority resistance variants.** The mean plasma HIV-1 RNA level of the 30 subjects evaluated was 4.6 $log_{10}$ (the median was 4.7), with a range of 2.6 to 7.4. Amino acid substitutions in PR and RT are shown in Table 1. Seven of these individuals appeared to be resistant to at least one PI and to at least one nucleoside RT inhibitor (NRTI) or NNRTI. One individual possessed the D30N and N88D mutations in PR, associated with resistance to nelfinavir. Minor resistance mutations were observed at positions 10, 33, 36, 63, 70, 71, 77, and 93 in PR. In regard to RT, two and four subjects were resistant to at least one NRTI and one NNRTI, respectively. The thymidine-associated mutations included M41L, L210W, and T215Y, while V118I, L210F/M, and G333E were observed in some samples. One sample (specimen 2) appeared to be resistant to both some NRTIs and some PIs.

The results (Table 1) show that K103N and M184V minority species were found in three and four patients, respectively. One sample that was found to contain M184V by AS-PCR (sample 12) did not contain other detectable RT resistance mutations. In contrast, other samples, e.g., numbers 1, 10, and 23, contained other NRTI or NNRTI resistance mutations as determined by bulk sequencing.

No major resistance mutations were found in any of the viruses (samples 18, 21, and 29) that contained K103N minority species. All these patients had high viral loads (mean plasma viral load, 4.3 ± 0.5 $log_{10}$ copies/ml).

In summary, we studied the prevalences of the K103N and M184V mutations in a group of 30 PHI patients who showed resistance to at least one drug as determined by conventional resistance testing. Circulation of such resistant strains can result in impairment of responsiveness to first-line therapy (2). Using an AS-PCR approach, we have now shown that both the K103N and the M184V mutations were detectable as minority variants in acute seroconverters, regardless of the low prevalence of the M184V mutation in such individuals as observed by bulk sequencing (12). The K103N mutation, in contrast to M184V, has only a low impact on replicative fitness (1, 9). In the absence of drug pressure, viruses harboring M184V can

rapidly revert to the wild type or deselect this mutation and, consequently, are less well detected by bulk sequencing (12). One recent report found an association between the presence of baseline minority resistance variants and nonresponsiveness to highly active antiretroviral therapy (5), although other studies have not obtained similar results (8, 10).

Although M184V-containing viruses can rapidly revert to the wild type, the persistence of M184V-containing strains is of concern in the management of antiretroviral therapy. Despite the impact of M184V on HIV fitness, our results document that this mutation can be detected in acutely infected, ARV-naive patients, suggesting that transmission of this mutation takes place at a higher frequency than suggested by the results of bulk sequencing (12).

**Nucleotide sequence accession numbers.** The sequences described in this study have been deposited in GenBank under accession numbers EU375800-801 and EU 906882-907.

This work was sponsored by the Canadian Institutes of Health Research (CIHR).

Thomas d'Aquin Toni is the recipient of a CIHR postdoctoral fellowship award. Michel Ntemgwa is the recipient of a CIHR doctoral fellowship award.

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