HIV-1 Drug Resistance Genotype Results in Patients With Plasma Samples With HIV-1 RNA Levels Less Than 75 Copies/mL

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Summary: HIV-1 genotypic resistance test results were obtained on clinical samples from 116 patients with plasma HIV-1 RNA levels of less than 75 copies/mL. Genotype validity was confirmed in 49 of 50 patients with a previous or follow-up genotype. The belief that genotypic resistance testing is unreliable in samples with low-level viremia should be reassessed.

Key Words: genotypic testing, population-based sequencing, low viral load

(*J Acquir Immune Defic Syndr* 2006;43:56–59)

In patients whose virus levels are suppressed to undetectable levels, as measured with current quantitative assays, the risk of virologic rebound, drug resistance, and disease progression is significantly lower than in patients with persistently detectable plasma viremia.[1–5] However, according to the major US treatment guidelines, there is a significant gap between the level of viremia indicative of virologic failure (>50 to >400 copies/mL) and the level of viremia at which genotypic resistance testing is recommended (>500 to 1000 copies/mL).[6–8] The explanation for this gap is a concern that genotypic resistance testing is difficult to perform and may not be reliable when plasma HIV-1 RNA levels are less than 1000 copies/mL.[6,8]

Two laboratories have recently reported amplifying and sequencing plasma HIV-1 protease and reverse transcriptase (RT) from 84% and 89% of 112 and 144 clinical plasma samples, respectively, with plasma HIV-1 RNA levels between 50 to 1000 copies/mL.[9,10] During the past 8 years, our clinical laboratory has often obtained reproducible genotypic test results on plasma samples with RNA levels that were below the level of quantification (BLQ) using either the Amplicor (Roche Molecular Diagnostics, Branchburg, NJ) or Versant (bDNA; Bayer Diagnostics, Emeryville, CA) assays. Here, we describe this phenomenon in a single clinical population, providing evidence for its validity and describing the clinical scenarios in which it occurs.

Received for publication February 13, 2006; accepted May 23, 2006.

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METHODS AND RESULTS

Patients, Samples, Plasma HIV-1 RNA Levels, and Genotypes

Three thousand six hundred thirty-one genotypic resistance tests and plasma HIV-1 RNA levels were performed on samples from 2697 patients of 16 California Kaiser-Permanente Medical Care Program clinics between July 1998 and August 2005. Of these 3631 tests, 122 (3.4%) from 116 persons were performed on plasma samples that had virus levels BLQ (<75 copies/mL) of the Versant HIV-1 RNA 3.0 assay. Virus load tests were performed at a Kaiser-Permanente Medical Care Program reference laboratory, and genotypic resistance tests were performed at Stanford University Hospital. Genotypic resistance tests were performed as previously described.[11] Briefly, RNA was extracted from 0.2 to 0.5 mL of plasma using a guanidine thiocyanate lysis reagent. Complement DNA was generated from viral RNA, and first-round polymerase chain reaction (PCR) was performed using Superscript One-Step RT-PCR (Life Technologies, Rockville, MD). Nested PCR was used to amplify a 1.3-kb product encompassing the protease gene and the first 300 residues of the RT gene. Direct PCR cycle sequencing was performed with AmpliTaq DNA FS polymerase and dRhodamine terminators (Applied Biosystems Inc, Foster City, CA).

To exclude the possibility that the undetectable bDNA measurements were false-negative values resulting from technical artifacts, 20 of the 122 genotyped BLQ samples were retested using the Amplicor HIV-1 Monitor test. Of these 20 samples, 5 had undetectable RNA levels (<3–4 copies/mL), 8 had detectable RNA levels BLQ (<50 copies/mL), and 6 had levels between 50 and 120 copies/mL.

Fifty of the 116 patients with genotyped BLQ RNA samples also had additional genotypic results generated at a time when their plasma HIV-1 RNA levels were detectable (48 before our BLQ genotyping). To exclude the possibility that the BLQ RNA genotypes resulted from PCR contamination or sample mix-up, we performed a phylogenetic analysis

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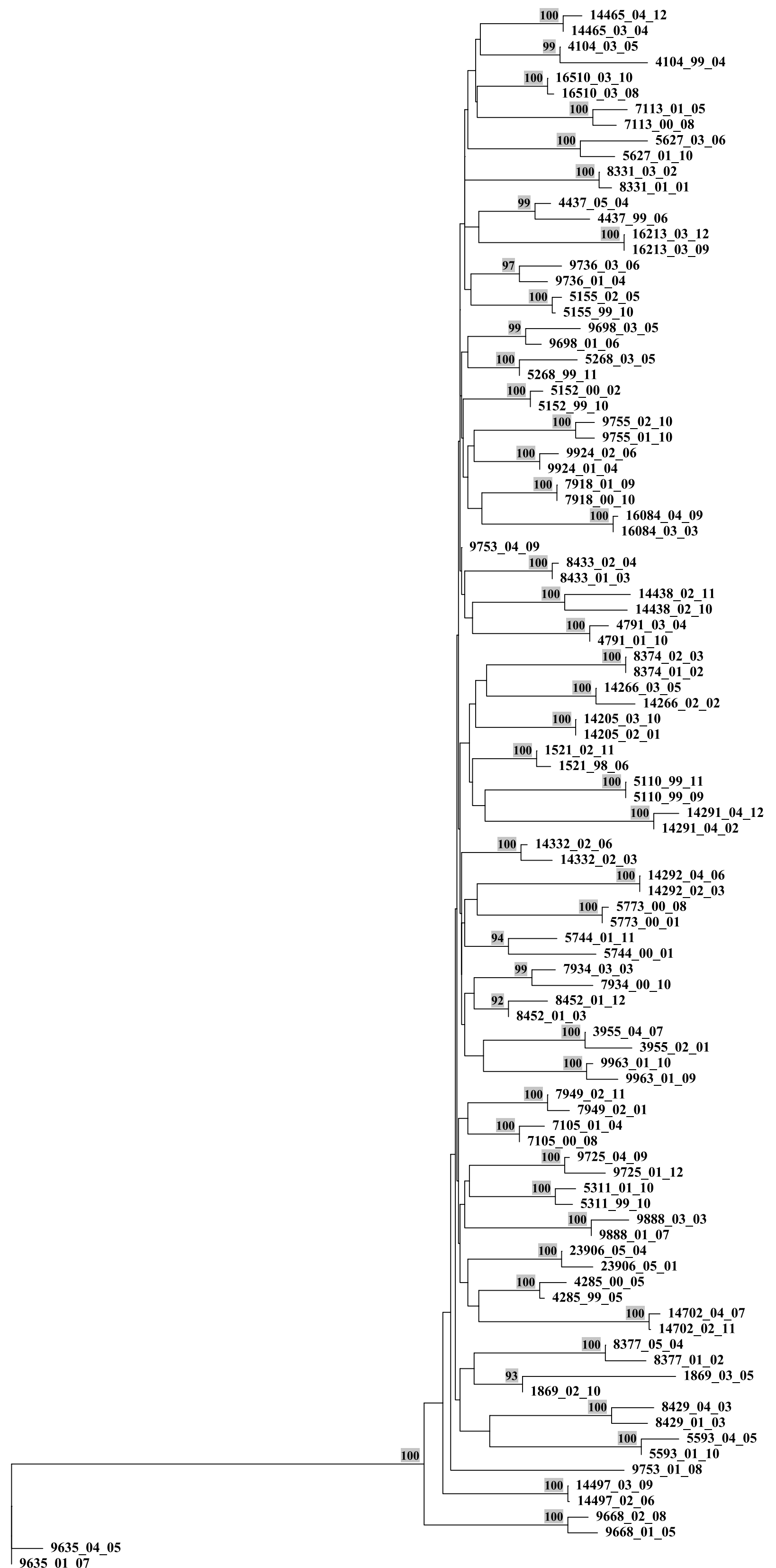

FIGURE 1. Neighbor-joining tree created using protease and RT nucleotide sequences from 50 patients with positive genotypic resistance tests on samples with plasma HIV-1 RNA levels less than 75 copies/mL and at least one additional genotypic resistance test performed at a time when RNA levels were detectable. Sequences from patient 9635 matched subtype C reference sequences and formed an out-group relative to the remaining subtype B sequences. Although 142 genotypes were performed on samples from these 50 patients (a mean of 2.8 genotypes per patient), only 2 genotypes per person are shown in this figure to enable it to fit on 1 page. The tree containing all 142 sequences also showed that sequences from 49 of the patients formed clades.

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of the *pol* sequences from the 50 patients with more than one available genotype. The mean mixture-weighted genetic distance[12] between sequences from the same patient was 1.5% (range, 0.1%–5.5%) compared with 5.1% (range, 2.9%–11.5%) between sequences from different patients. A neighbor-joining tree created from these sequences showed that the sequences of 49 of 50 patients formed a clade with bootstrap values more than 80% (100 replicates; Fig. 1). The 2 sequences from the patient that did not form a clade (and diverged by 5.5%) were excluded from further analyses.

Clinical Significance and Relationship to Antiretroviral Therapy

Treatment history and sequential plasma HIV-1 RNA levels were available for 45 of the 49 patients whose genotyped BLQ sample was confirmed through the formation of a clade with other genotypes from the same patient. In 20 of these 45 patients, the genotype and plasma HIV-1 RNA level tests were ordered when previously undetectable RNA levels rebounded to detectable levels. In 7 patients, the genotype and plasma HIV-1 RNA level tests were ordered when viral RNA levels declined slowly in response to a change in antiretroviral therapy. In 18 patients, the virus levels preceding the BLQ sample were persistently elevated suggesting that the BLQ result may have resulted from increased patient adherence before genotypic resistance testing.

In 23 of 45 patients with confirmed genotyped BLQ samples and available treatment history, subsequent RNA levels remained less than 75 copies/mL without a change in antiretroviral therapy. In 5 patients, subsequent RNA levels remained less than 75 copies/mL after a change in therapy was made in response to the genotype results. Subsequent RNA levels rebounded to detectable levels in 13 patients who continued the same regimen. Two patients discontinued therapy, and subsequent RNA levels were not available for 2 additional patients.

Sequences from each of the 45 patients were evaluated by the June 2005 version of the Stanford HIVdb genotypic resistance interpretation system (http://hivdb.stanford.edu). Among the 28 patients with sustained virologic suppression after the BLQ genotype, 12 had viruses with intermediate or high-level resistance to 1 (6 patients), 2 (4 patients), or 3 or more drugs (2 patients) received after the genotyped BLQ sample (as noted in the previous paragraph, 5 of these 28 patients changed therapy after the BLQ genotype).

DISCUSSION

This study shows that it is not unusual to sequence virus from plasma samples reported to have virus levels BLQ and demonstrates the validity of these sequence results in nearly all patients (49/50) in whom a previous genotype was available for comparison. The study was based on data from a single clinical population in which 122 (3.4%) of 3631 samples genotyped had plasma HIV-1 RNA levels below the lower limit of quantification of 75 copies/mL using the Versant HIV-1 RNA assay. However, we have observed the same phenomenon in 9 (2.1%) of 431 patients in a different clinical population in which the Amplicor HIV-1 Monitor assay was used (data not shown). Success in accurately genotyping samples with low levels of plasma viremia is consistent with the recent studies reporting sensitivities of 84% to 89% for samples with plasma HIV-1 RNA levels between 50 and 1000 copies/mL[9,10] and with a previous report from our laboratory using a similar sequencing method that reported a 50% probability of obtaining a sequence on samples with a plasma HIV-1 RNA level of 40 copies/mL.[13]

This study was not designed to assess the sensitivity of genotyping because unsuccessful attempts at sequencing were not routinely documented for most of the 8-year study period. A review of written records for the last 2 years of the study, however, revealed that 30 (27%) of 111 samples with RNA levels less than 75 copies/mL were successfully genotyped (data not shown). These results, however, do not provide a reliable assessment of sensitivity because the successfully genotyped BLQ RNA samples were nearly always obtained from persons with recent episodes of detectable viremia (which prompted the request for genotyping). Indeed, 2 clinical scenarios provided the context for more than half of the genotyped BLQ samples: patients with a transient virologic rebound and patients with a slow virologic response to a preceding change in antiretroviral therapy. In each case, the clinician's rationale for ordering a genotypic resistance test was to determine whether the virologic rebound or slow virologic response was caused by drug resistance that would require a change in therapy.

Although this study does not prove the clinical usefulness of genotypic resistance testing on samples with low-level viremia, our data in combination with other reports showing a high sensitivity of sequencing protease and RT in plasma samples with less than 1000 copies/mL suggest that the role of resistance testing in patients with virus that is detectable but less than 1000 copies/mL should be reassessed.

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