Presence of a Multidrug-Resistance Mutation in an HIV-2 Variant Infecting a Treatment-Naive Individual in Caio, Guinea Bissau

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We report the possible transmission of drug-resistant human immunodeficiency virus type 2. A 66-year-old woman from rural Guinea Bissau who had no obvious antiretroviral exposure was found to harbor a variant with the multidrug-resistance mutation Q151M. Finding this mutation among a drug-naive population presents an important public health issue that needs to be addressed for treatment to be effective.

Transmission of drug-resistant human immunodeficiency virus type 1 (HIV-1) has been reported in several developed countries, with prevalences ranging from 10% to 30% of HIV-1 infections [1, 2]. Frequent occurrences in the United States have led to new guidelines that propose the use of resistance testing for treatment-naive individuals before the initiation of therapy [3].

Increasing access to antiretrovirals in Africa has made studies of HIV type 2 (HIV-2) drug resistance a priority. Antiretrovirals were not officially available in Caio, Guinea Bissau, until 2007. In preparation for the initiation of therapy, 23 patients were randomly screened for the presence of drug-resistance mutations, to maximize the efficiency of the first-line regimen. We report here the presence of a multidrug-resistance mutation in an HIV-2 variant infecting a treatment-naive individual in Caio.

Methods. The Caio cohort was initiated to study the epidemiology of HIV-2 infection. Caio, located in the northwestern part of Guinea Bissau, is an isolated rural community that does not have running water or electricity. Three population-wide serosurveys (during 1989–1991, 1996–1998, and 2006–2007) have been conducted in Caio to determine the HIV status of its inhabitants. In 2003, some cohort members were enrolled in a case-control study. Each year, births, deaths, immigration into Caio, and emigration out of Caio were recorded. It was established that many of the male villagers mostly live and work in larger towns in the subregion or in Portugal or France and that a substantial number of the women work or have worked as commercial sex workers in the capital city, Bissau, or in neighboring countries [4]. The prevalence of HIV-1 infection increased gradually (from 0.5% during 1989–1991 to 2.7% during 1996–1997 to 3.6% during 2006–2007), whereas the prevalence of HIV-2 infection decreased (from 8.3% during 1989–1991 to 7.9% during 1996–1997 to 4.7% during 2006–2007). Between each serosurvey, HIV-infected individuals were followed up more regularly and had free access to clinical care. All study participants in the cohort were antiretroviral therapy naive and provided informed consent. Results of HIV testing were made available on request; most participants did not seek their results. The joint Gambian Government–Medical Research Council Ethics Committee and the National AIDS Control Programme Committee of Guinea Bissau approved this study.

CD4 T cells were quantified by flow cytometry, and plasma viral load (in HIV-2 RNA copies per milliliter) was measured by an in-house assay, as described elsewhere [5]. HIV-2 RNA or DNA was amplified and directly sequenced as described elsewhere [5]. The sequences generated were assigned GenBank accession numbers AM408185–AM408208 and FM877561. Virus was analyzed by 2 genotypic methods, sequencing and the oligonucleotide ligation assay (OLA). The HIV-2 OLA for the Q151M and M184V mutations has been described elsewhere [5].

Results. Phylogenetic analysis showed the HIV-2 samples from all patients to be subtype A. Of the 23 patients whose virus was genotyped, 22 were found to be infected with wild-type HIV-2. As has been observed for other HIV-2 wild-type samples [6], some HIV-1 drug-resistance mutations in protease (L10V, K20R, L33V, V32I, M36I, M46I, I47V, I62V, A71V, G73A, and I93L [data not shown]) and reverse transcriptase (V75I

Received 24 November 2008; accepted 22 January 2009; electronically published 13 May 2009.

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Clinical Infectious Diseases 2009; 48:1790–3

1058-4838/2009/4812-0027$15.00
DOI: 10.1086/599107

and K219Q/E [table 1]) were found as natural polymorphisms in the HIV-2 samples analyzed here. In virus from 1 patient, a multi–nucleoside reverse-transcriptase inhibitor (NRTI)–resistance mutation, Q151M, was discovered. Two subsequent samples from this patient were also genotyped, and this mutation was present in both (table 1). The sequencing and OLA results were concordant.

The index patient (Caio 12) is a 66-year-old woman from Caio who at some point had worked as a commercial sex worker in the capital city, Bissau. In 1989, during the first serosurvey, the patient tested negative for both HIV-1 and HIV-2; however, she tested positive for HIV-2 in 1997, during the second serosurvey. In 2003, the index patient was still infected with HIV-2 only, had a plasma viral load of 37,427 copies/mL, and had a CD4 T cell count of 123 cells/mL (CD4 T cell percentage, 11%). In 2006, the last serosurvey showed that the patient was still singly infected with HIV-2 and had a plasma viral load of 25,836 copies/mL and a CD4 T cell count of 130 cells/mL (CD4 T cell percentage, 9%). Clinical examination in 2006 showed that the patient had evidence of HIV disease (axillary, mandibular, and inguinal lymphadenopathy).

During each serosurvey, the cohort members were administered 2 questionnaires, 1 by a fieldworker and 1 by a physician (to determine clinical history), who also conducted a clinical examination. In both, past or present use of antiretrovirals was queried. Also, queries about general drug use were made: cohort members were asked whether they were receiving long-term treatment and, if so, what the drugs were, where they came from, and whether they were from relatives in Europe or big cities. If a patient did not know the purpose of the drugs he or she was using, a fieldworker would be sent to the patient's home to record what the drugs were. During these serosurveys, no antiretroviral use was recorded, and the replies did not suggest that any of the HIV-positive patients were receiving antiretroviral therapy.

The index patient has been followed up since 1989 but had never requested to see her HIV testing results. During the 2006 clinical examination, the physician and fieldworker noted that the patient was unaware of her HIV status. However, when antiretrovirals become available in Caio at the end of 2007, the patient was counseled, informed of her HIV status, and offered drugs, but she did not want to start treatment as yet. First-line antiretroviral therapy in Caio is zidovudine, lamivudine, and nevirapine for HIV-1 infection and zidovudine, lamivudine, and indinavir/ritonavir for HIV-2 infection.

All 3 available samples from this patient (from 1997, 2003, and 2006) have been genotyped, and the presence of the Q151M mutation—which is associated with phenotypic resistance to the NRTIs zidovudine, didanosine, zalcitabine, abacavir, and stavudine in both HIV-1 and HIV-2 infection [7]—was revealed in all of them. The results of the questionnaires and the fact that the index patient did not seem to know her HIV status at the time the samples were obtained seem to indicate that she has never been treated, which thereby suggests that the she might have been infected with a drug-resistant strain of HIV-2.

Discussion. For HIV-2 infection, limited treatment options resulting from natural resistance has made choosing a sufficiently potent and durable first-line regimen even more important. Widespread use of antiretrovirals for the treatment of HIV-1 infection has led to an increase in the transmission of drug-resistant viruses. Consequently, it is important to screen treatment-naive patients who are about to initiate therapy [8].

Although the precise time of seroconversion cannot be determined for the index patient, several factors indicate that the presence of the Q151M mutation is most likely the result of transmission of drug-resistant virus and not of prior drug exposure.

Drug-resistance mutations can occur by natural evolution or can be selected in the presence of drug pressure. However, primary mutations, which cause high-level resistance, are not expected to occur naturally in treatment-naive individuals, because the acquisition of primary mutations is, with a few exceptions, usually associated with a significant loss in fitness in the absence of drug pressure [9, 10]. Therefore, for untreated patients the presence of primary mutations is believed to indicate transmission of drug-resistant HIV, whereas the presence of secondary mutations is associated with natural polymorphisms [11]. The Q151M mutation has not been observed as a natural polymorphism in any of the known HIV variants; its presence has been associated solely with NRTI treatment.

Q151M is a primary resistance mutation that is associated with multi-NRTI resistance in both HIV-1 and HIV-2 infection. Although rare in HIV-1 infection, it occurs more frequently in HIV-2 infection [7]. Although viruses with primary mutations are usually less fit than their wild-type counterparts, Q151M mutants are among the few exceptions—the presence of Q151M in fact increases the fitness of the mutant virus [10]. The improved fitness of Q151M mutants may explain the apparent persistence and stability of this mutation in our patient. Also, it has been demonstrated that, unlike in the setting of treatment interruption, transmitted drug-resistant viruses can persist for many years [8]. During transmission of drug-resistant viruses, these resistant variants represent the majority of the quasispecies, and in most cases wild-type viruses will not be transmitted. Hence, no latent or archived wild-type viruses will be present to later outcompete the transmitted drug-resistant viruses [9, 12].

That antiretrovirals have not been widely used in West Africa and were not available in Caio until 2007, coupled with the fact that the patient was unaware of her HIV status, seem to indicate that the index patient had never received antiretro-

Table 1. Amino acids in human immunodeficiency virus type 2 (HIV-2) samples from patients in the Caio cohort, by known human immunodeficiency virus type 1 (HIV-1) nucleoside reverse-transcriptase inhibitor (NRTI) mutations.

Category	Sample type	GenBank accession no.	Common mutations in HIV-1–infected patients exposed to NRTIs															
			M41L	K65R	D67N	T69D	K70R	L74V	V75I	F77L	Y115F	F116Y	Q151M/L	M184V	L210W	T215Y/F	K219Q/E	G333D/E
Wild-type virus																		
HIV-1	…	K03455	M	K	D	T	K	L	V	F	Y	F	Q	M	L	T	K	G
HIV-2	…	M15390	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Patient sample																		
Caio 1	DNA	AM408185	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 3	DNA	AM408186	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 6	RNA	AM408187	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 7	RNA	AM408188	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 8	DNA	AM408189	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 9	RNA	AM408190	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 10	RNA	AM408191	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 11	RNA	AM408192	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 12.0[a]	DNA	FM877561	M	K	D	N	K	L	*I*	F	Y	F	**M**	M	N	S	*E*	Q
Caio 12[a]	RNA	AM408193	M	K	D	N	K	L	*I*	F	Y	F	**M**	M	N	S	*E*	C
Caio 12.1[a]	RNA	AM408194	M	K	D	N	K	L	*I*	F	Y	F	**M**	M	N	S	*E*	Q
Caio 13	DNA	AM408195	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 14	RNA	AM408196	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 15	DNA	AM408197	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 17	DNA	AM408198	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 19	DNA	AM408199	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 20	DNA	AM408200	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 21	DNA	AM408201	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 22	DNA	AM408202	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 24	DNA	AM408203	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 25	DNA	AM408204	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 26	DNA	AM408205	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 27	DNA	AM408206	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 28	DNA	AM408207	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q
Caio 29	DNA	AM408208	M	K	D	N	K	L	*I*	F	Y	F	Q	M	N	S	*E*	Q

NOTE. Resistance mutations are shown in boldface type. Human immunodeficiency virus type 2 (HIV-2) polymorphisms that can affect drug susceptibility shown are in italics.

[a] Samples from the index patient.

virals. In addition, responses to questions about antiretroviral use suggest that the patient did not have prior antiretroviral experience. Furthermore, the remoteness of Caio and the lack of the most basic necessities in the community argue against possible antiretroviral use. However, it cannot be completely ruled out, given that individual and unauthorized antiretroviral use has been known to occur in some African countries. Regardless, the presence of a multi-NRTI–resistance mutation in a patient initiating therapy, whether the result of prior drug exposure or of transmission of drug-resistant virus, is of major public health concern. This finding in West Africa, where there is a large population of HIV-2–infected individuals who have few treatment options, provides strong support for the routine use of genotypic resistance tests for new patients starting therapy, to optimize the first-line regimen and allow for more-effective treatment.

Acknowledgments

We extend special thanks to Dr. Andrew Wilkins, Dr. Dominique Ricard, and Margaret Buckner for setting up the Caio cohort. We acknowledge the population of Caio, Guinea Bissau, for participating in this study; the team of Caio field assistants; and the staff of the genitourinary medicine clinic in Fajara, The Gambia, for sample collection. We are grateful to Matthew Cotton, Chris Parry, and Deenan Pillay for critical review of the manuscript.

Financial support. This work was partially funded by the Medical Research Council (United Kingdom). S.J. was supported by an Institute of Tropical Medicine/Belgian Directorate General for Development Cooperation doctoral scholarship from the Institute of Tropical Medicine in Antwerp, Belgium.

Potential conflicts of interest. All authors: no conflicts.

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