CASE SERIES

Transmitted and Acquired HIV-1 Drug Resistance from a Family: A Case Study

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Abstract: Antiretroviral drug resistance has become a major threat to the adequate management of human immunodeficiency virus (HIV) infection, but little attention has been paid to the spread and evolution of drug-resistant strains in the family. Here, we described a case of transmitted as well as acquired HIV drug resistance among a father, mother and infant. Epidemiological data were obtained retrospectively. Drug resistance mutations (DRMs) of three patients were tested using a validated In-house Sanger-based sequencing (SBS) method and the Vela next-generation sequencing (NGS) platform. Gene evolution analysis was also performed. According to the epidemiological history and phylogenetic data, in late pregnancy of the mother, the infant's father transmitted HIV-1 to her, and then the mother to the baby, leading to the transmission of V106I as a common mutation of three persons. The mutant frequency was 99.57% (father), 95.38% (mother) and 99.73% (infant), respectively. Mother also acquired K101E (41.03%), K103N (27.56%) and minor mutation of V106M (4.30%) after improperly discontinuing antiretroviral regimen of lamivudine (3TC), tenofovir (TDF) and efavirenz (EFV). Such acquired mutations increased the drug resistance scores on non-nucleoside reverse transcriptase inhibitors (NNRTIs) doravirine, EFV, etravirine, nevirapine and rilpivirine from 10, 0, 10, 10 and 10 to 65, 135, 25, 150 and 55, respectively. Therefore, sexually transmitted diseases, especially DRMs of HIV-1 in families, are of concern and draw attention to the need for enhanced drug-resistance prevention efforts, and accurate surveillance by more sensitive methods in complicated cases.

Keywords: HIV-1, drug resistance, minor mutation, sexually transmitted diseases, next-generation sequencing

Introduction

The emergence of drug resistance has become a very real threat to the adequate management of human immunodeficiency virus (HIV) infection worldwide.[1,2] Drug resistance mutation (DRM) is acquired due to viral replication and selective pressure in patients receiving antiretroviral therapy (ART), but can be transmitted to drug naïve persons newly infected with HIV.[3,4] In the case of pregnant women, the mother-to-child transmission of DRM strains can complicate the infant management.[5]

All current antiretroviral drugs are at risk of emerging DRM.[1] For the commonly used alternative first-line regimen based on the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV) in combination with two nucleoside reverse transcriptase inhibitors (NRTIs), usually tenofovir (TDF) and either lamivudine (3TC) or emtricitabine,[6] DRM is more common in EFV.[1,7] A major disadvantage of EFV is the low genetic barrier that certain single amino acid mutation, such as K103N,

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V106M, can cause a high level of drug resistance.[8] Furthermore, poor adherence leads to insufficient medication exposure and selection pressure of EFV, which increases the risk of DRM.[9] The plasma terminal half-life of 3TC and TDF are approximately 5 to 7 hours and 17 hours, respectively,[10,11] while EFV averages 52 to 76 hours after a single dose and 40 to 55 hours after multiple doses,[12] and even 136 hours in some individuals.[13] Long half-life thus results in EFV monotherapy with more rapidly clearance of other drugs after treatment interruption and causes a high-risk context for the emergence of DRM.

Importantly, development of DRM can reduce the virological response,[14] increase the probability of transmission of resistant viruses to people newly infected with HIV,[15,16] and even lead to multidrug resistance in naïve patients.[17] Therefore, genotyping resistance testing is recommended as a routine laboratory monitoring before starting ART.[18,19] Sanger-based sequencing (SBS), the most commonly used method to diagnose HIV drug resistance,[20] was reported to have high repeatability and interpretability.[21] However, only virus strains with mutation frequency more than 10% to 20% can be detected by direct sequencing,[22] and the minority variants missed by standard genotyping may be lead to viral failure.[23–25] Thus, more sensitive methods of drug resistance testing are necessary to detect minority variants, so as to better guide clinical decision-making.

Here, we reported a case of transmitted HIV-1 drug resistance from a family as well as acquired drug resistance after improper discontinuation of 3TC, TDF and EFV, and analyzed the application of Vela next-generation sequencing (NGS) platform in detection of minority variants in complex cases.

Case Description

Patient M (Infant's mother), a 27-year-old woman, was tested HIV-negative at 12 and 32 weeks of gestation, respectively (Figure 1), but was suspected HIV-positive in a local hospital on July 7, 2019 (one day before delivery) and was confirmed five days after childbirth by local Centers for Disease Control. She had no sexual history before marriage and also had no history of intravenous drug use, surgical trauma or blood transfusion. She and her husband did not screen for sexually transmitted diseases (STDs) before pregnancy but had unprotected sexual contact at 32 weeks of gestation. Patient M stopped breastfeeding after the diagnosis and initiated on a first-line regimen of 3TC, TDF and EFV on September 15, 2019. However, she took only eight days of antiviral drugs and stopped them simultaneously because of a severe systemic
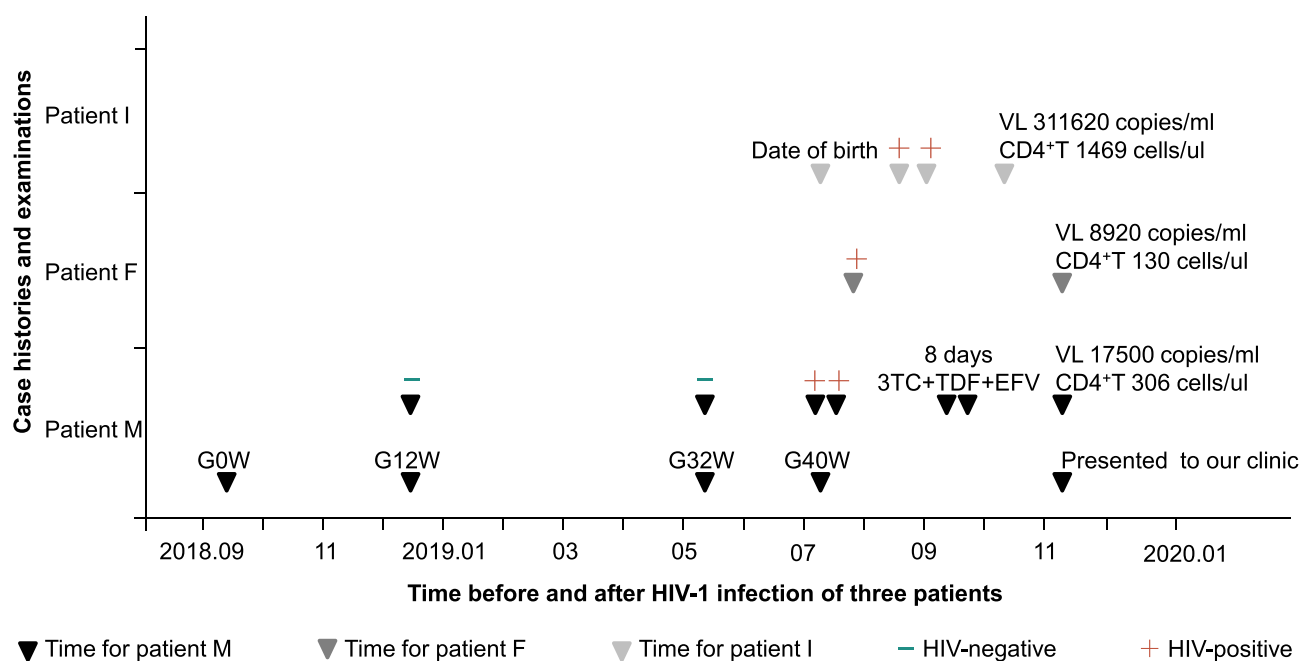

Figure 1 Summary of the case.
Abbreviations: Patient M, Infant's mother; Patient F, Infant's father; Patient I, Infant; G0W, 0 weeks of gestation; G12W, 12 weeks of gestation; G32W, 32 weeks of gestation; G40W, 40 weeks of gestation; HIV-Ab, human immunodeficiency virus antibody; ART, antiretroviral therapy; VL, viral load; 3TC, lamivudine; TDF, tenofovir disoproxil fumarate; EFV, efavirenz.

skin allergy. Then, she presented to our clinic on November 12. The viral load (VL) was tested to be 17,500 copies/mL and CD4$^+$T was 306 cells/ul.

Her contact, Patient F (Infant's father) was a 30-year-old man and had a history of sexual contact with other female before marriage. However, he was confirmed HIV-positive for the first time after patient M was diagnosed. Baseline bloods showed VL 8920 copies/mL and CD4$^+$T 130 cells/ul.

Their infant, patient I, was born naturally at full term on July 8, 2019. He was found to be positive for HIV antibody at 42 and 53 days after birth, respectively. Then, presented to our clinic on October 10. Baseline bloods showed VL 311,620 copies/mL and CD4$^+$T 1469 cells/ul.

Methods
Data Source and Genotype Analysis
Clinical data and blood samples were obtained between October 10 and November 12, 2019. Genotypic resistance mutation analysis was performed using a validated In-house SBS method. In this reaction, the entire protease gene and the first 300 codons of reverse transcriptase gene, as well as the entire integrase gene were amplified, respectively. Stanford HIV-1 drug resistance database (HIVdb version 8.9–1) was used to analyze the mutations and generate the final reports including drug resistance scores, with 0–9 for susceptible, 10–14 for potential low-level resistance, 15–29 for low-level resistance, 30–59 for intermediate resistance, and ≥ 60 for high-level resistance.

Then, the Vela NGS-based HIV-1 DRM test was also performed. HIV RNA was extracted from plasma sample and was used for one-step reverse transcription-polymerase chain reaction. The amplicons were enzymatically fragmented and subjected to NGS on the Sentosa® SQ301 sequencing instrument. Subsequently, Sentosa® SQ Suite software performed primary analysis on the raw sequencing data and then transferred to Sentosa® SQ Reporter Server for secondary analysis and report generation.

Gene Evolution Analysis
Homologous sequence comparison was analyzed by Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov) using HIV-1 *pol* gene obtained by In-house method. And these *pol* sequences from three patients were uploaded to the GenBank website (https://www.ncbi.nlm.nih.gov/genbank/; Accession numbers: MT939518, MT939519 and

MT939520, respectively). The reference sequences of other subtypes were downloaded from Genbank. Phylogenetic tree was constructed using MEGA (Version 7.0, USA) software and the between group mean distance as well as bootstrapping test were performed.

Results
Three Patients Had a Common Transmitted Mutation of V106I
Patient I was firstly performed DRM testing using SBS method on October 14 before starting ART and was found to be subtype B infection and V106I mutation (Table 1). Then, patient M and patient F were performed DRM testing on November 12. Both of them were subtype B and had no resistance mutation to NRTIs, protease inhibitors or integrase inhibitors, but had a common V106I mutation. Patient F also had an E138A mutation. Subsequently, the DRMs were confirmed using Vela NGS method and the mutant frequency of V106I for three patients were 99.73% (Patient I), 95.38% (Patient M) and 99.57% (Patient F), respectively. The mutant frequency of E138A was 67.78% and was not transmitted to patient M.

To determine the evolutionary relationship of HIV strains in three patients, phylogenetic tree analysis were performed (Figure 2). These three patients were shown to be subtype B and the genetic distance among them were 0.0017 (mother and infant), 0.0075 (mother and father) and 0.0083 (father and infant), respectively. Moreover, the virus they infected came from a homologous strain and the percent identity was 97.33% (patient M), 97.52% (Patient I) and 97.94% (Patient F). Surprisingly, the homologous strain itself had V106I mutation according to the Stanford HIV-1 drug resistance database.

Patient M Acquired K101E, K103N and Minor Mutation of V106M After Improperly Discontinuing Antiretroviral Drugs
Patient I and patient F tested for DRM before the initiation of ART. However, patient M took eight days' first-line regimen (3TC, TDF and EFV) two months before presented to our clinic. In addition to the transmitted drug resistance of V106I mutation, she acquired K101E and K103N mutations with mutant frequency of 41.03% and 27.56%, respectively. Subsequently, the sensitivity comparison of In-house SBS and Vela NGS methods to detect minority variants of HIV-1 DRM was performed. As it turned out, a minor V106M mutation with a frequency of

Table I Genotype Analysis of Drug Resistance Mutation

Parameters	Patient I (Infant)		Patient M (Mother)		Patient F (Father)	
	SBS	Vela NGS	SBS	Vela NGS	SBS	Vela NGS
Drug resistance interpretation: PR						
PI Resistance Mutations	None	None	None	None	None	None
Drug resistance interpretation: IN						
IN Resistance Mutations	None	None	None	None	None	None
Drug resistance interpretation: RT						
NRTI Resistance Mutations	None	None	None	None	None	None
NNRTI Resistance Mutations	V106I	V106I (99.73%)	V106I K101E K103N	V106IM (I95.38%, M4.30%) K101E (41.03%) K103N (27.56%)	V106I E138A	V106I (99.57%) E138A (67.78%)
Non-nucleoside reverse transcriptase inhibitors:						
Doravirine (DOR)	P	P	L	H	P	P
Efavirenz (EFV)	S	S	H	H	S	S
Etravirine (ETR)	P	P	L	L	L	L
Nevirapine (NVP)	P	P	H	H	P	P
Rilpivirine (RPV)	P	P	I	I	L	L

Abbreviations: SBS, Sanger-based sequencing; NGS, next-generation sequencing; PR, protease; PI, protease inhibitor; IN, integrase; RT, reverse transcriptase; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; S, susceptible; P, potential low-level resistance; L, low-level resistance; I, intermediate resistance; H, high-level resistance.

4.30% was found by NGS platform in patient M but not identified by SBS method.

Acquired Mutations Aggravate the Drug Resistance Scores of Patient M

These five DRMs have varying degrees of effect on NNRTIs based on the mutation scores (Table 2). V106I mutation shows susceptible to EFV (0), and potential low-level resistance to doravirine (DOR, 10), etravirine (ETR, 10), nevirapine (NVP,10) and rilpivirine (RPV, 10), which increases to low-level resistance to ETR (20) and RPV (25) when E138A is also present. However, for patient M, V106M alone is associated with a high-level reduction in NVP (60) and EFV (60) susceptibility as well as an intermediate reduction in DOR (50) susceptibility, while together with V106I, especially K101E and K103N, the mutation scores are increased to 65, 135, 25, 150 and 55 for DOR, EFV, ETR, NVP and RPV.

Discussion

Antiretroviral drug resistance becomes a major threat for the adequate management of HIV infection.[2] Resistant mutation in the HIV genome results in hard-to-treat infections, increases health-care costs,[1] and can be transmitted from person to person,[26] as described in this case. Uniquely, for the first time, we proposed the necessity of screening for STDs in both couples at the same time during pregnancy testing and also emphasized the importance of monitoring for minor mutations in complex cases by NGS method.

Currently, the guidelines recommend that pregnant women should screen for STDs at the first prenatal visit, including HIV, a serologic test for syphilis, hepatitis B surface antigen, hepatitis C antibodies and Neisseria gonorrhoeae,[27,28] while no recommendation for simultaneous screening for sexual partners. However, STDs can also occur during pregnancy as well as lead to intrauterine or perinatal transmission. In this case, patient F was infected with the V106I mutant of HIV-1 according to phylogenetic tree analysis and developed to chronic infection status without knowing it. He transmitted the drug-resistant mutant viruses to patient M because he did not screen for STDs before or after his wife became pregnant. To make matters worse, the HIV programs to prevent mother-to-child transmission have failed, probably mainly due to the limitations of local medical conditions. This leads to the spread of HIV-1 mutant strains in this family, and causes a serious public health problem, which has far-reaching consequences for the long-term health and health care costs.[29] But worse, the overall incidence of STDs has been increasing in more recent years.[30,31] In China, an average of more than 1 baby per hour was reported to be
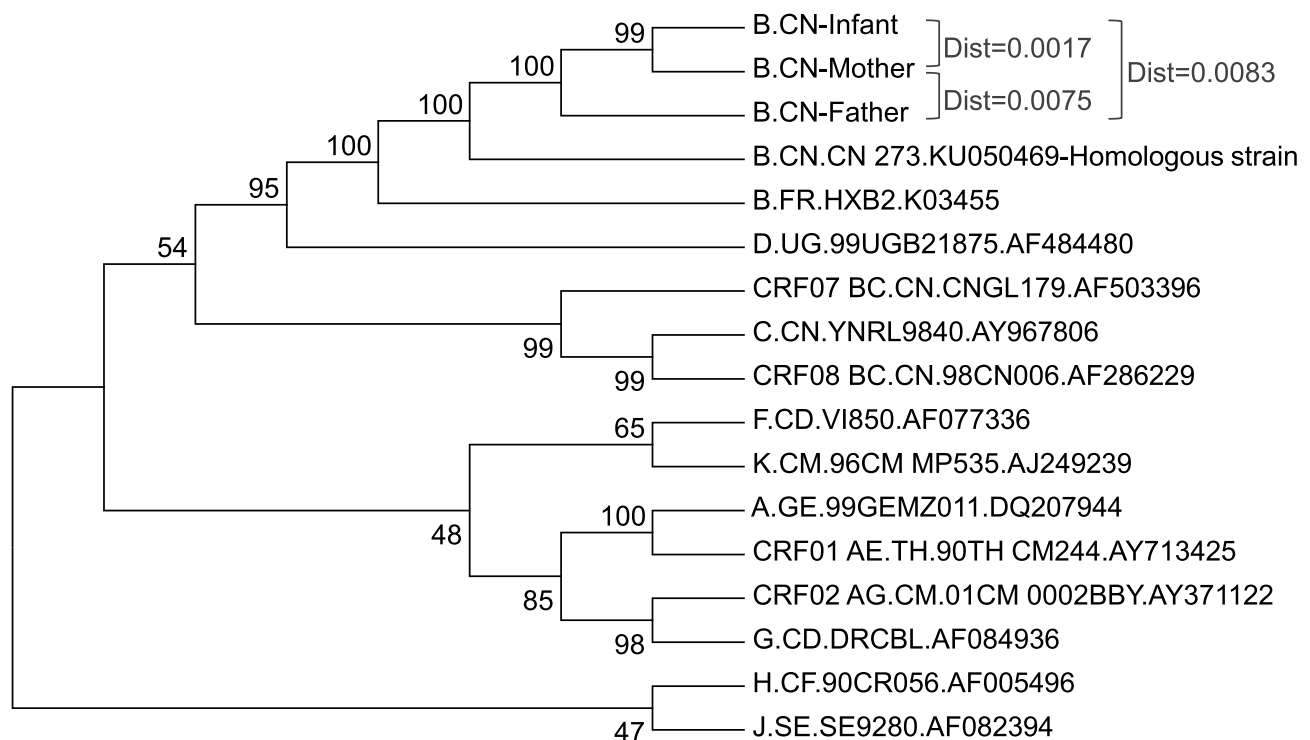
Figure 2 Gene evolution analysis of patients.
Abbreviation: Dist, distance.

born with congenital syphilis in 2008, for a total of 9480 cases.[32] Therefore, the case we described maybe just the epitome of many families with STDs.

The common V106I mutant of three patients, transmitted through sexual transmission and mother-to-child

Table 2 Mutation Scores of Non-Nucleoside Reverse Transcriptase Inhibitors

Patient	NNRTI	DOR	EFV	ETR	NVP	RPV
Patient I	V106I	10	0	10	10	10
	Total	10	0	10	10	10
Patient F	V106I	10	0	10	10	10
	E138A	0	0	10	0	15
	Total	10	0	20	10	25
Patient M (SBS)	V106I	10	0	10	10	10
	K101E	15	15	15	30	45
	K103N	0	60	0	60	0
	Total	25	75	25	100	55
Patient M (Vela NGS)	K101E	15	15	15	30	45
	K103N	0	60	0	60	0
	V106IM	60	60	10	70	10
	Total	65	135	25	150	55

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; DOR, doravirine; EFV, efavirenz; ETR, etravirine; NVP, nevirapine; RPV, rilpivirine; SBS, Sanger-based sequencing; NGS, next-generation sequencing.

transmission, respectively, is also a strong evidence of how DRM spreads in HIV-1 naïve individuals. In contrast to transmitted drug resistance, acquired drug resistance develops because of viral replication and selective pressure in patients receiving ART.[1,4] Patient M took eight days of 3TC, TDF and EFV and stopped three drugs simultaneously because of a severe systemic skin allergy, which may result in EFV monotherapy due to its longer half-life.[10–12] As expected, she rapidly acquired K101E, K103N and V106M mutations.

The proportion of wild type virus, and the resistant viruses evolved under the sustained selective pressure of drugs is different in the presence and absence of ART.[33] Persistent viremia in the presence of therapy leads to further accumulation of mutations and reduces replication of the wild type virus, which increases resistance.[4] However, the discontinuation of ART when viral suppression was not achieved causes a relatively fast decay of mutants, and the rapid re-emergence of wild type virus for their higher fitness or replication capacity without drug selective pressure.[34,35] Patient M performed genotypic resistance mutation testing two months after the cessation of ART, when the circulating viruses may have changed, so the mutants frequency of K101E and K103N were 41.03% and 27.56%, respectively. Long detection time interval after drug withdrawal may even

cause resistant strains to decline to levels undetectable by conventional In-house SBS approach. It must be noted, however, such minor mutant can also contribute to the failure of salvage regimens.[24,36,37] Consequently, we compared the sensitivity of In-house SBS and NGS methods to detect minority variants of HIV-1 DRM, and a minor V106M mutation with a frequency of 4.30% was found by NGS method in patient M but not identified by SBS, which is associated with high-level reductions in NVP and EFV susceptibility.

Our study also supports the recommendation of WHO guidelines that dolutegravir together with two NRTIs should be the preferred first-line regimen for HIV-infected adults initiating ART,[38] especially in resource limited settings. For the reason that resistance to NRTIs and integrase inhibitors was relatively stable, while resistance to NNRTIs increased significantly in the past few years.[1] However, in combination with our recommendations, their own economic conditions and local health insurance policies, patient M chose the national free antiviral drugs 3TC/TDF/lopinavir and ritonavir, patient F chose elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide regimen together with compound sulfamethoxazole for $CD4^+T$ was 130 cells/ul, patient I was given 3TC/abacavir/lopinavir and ritonavir regimen to return to the local home for treatment.

Conclusion

In summary, simultaneous screening of STDs for both husband and wife during pregnancy testing is necessary. Besides, in some complicated cases like intermittent ART, the Vela NGS platform has an advantage in identifying minority variants of HIV-1 drug resistance than the In-house SBS method.

Abbreviations

HIV, human immunodeficiency virus; DRM, drug resistance mutation; SBS, Sanger-based sequencing; NGS, next-generation sequencing; 3TC, lamivudine; TDF, tenofovir; EFV, efavirenz; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; ART, antiretroviral therapy; STDs, sexually transmitted diseases; VL, viral load; DOR, doravirine; ETR, etravirine; NVP, nevirapine; RPV, rilpivirine; PR, protease; PI, protease inhibitor; IN, integrase; RT, reverse transcriptase.

Ethics and Consent

This study was part of a research project that had been approved by the ethics committee of Beijing Ditan

Hospital of Capital Medical University (Approval number: 2019-037-002). Two adult patients provided written informed consent and signed consent for their infant, and they agreed to publish details of the three cases.

Disclosure

The authors report no conflicts of interest in this work.

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