

# Genotype and Phenotype Patterns of Drug-Resistant HIV-1 Subtype B' (Thai B) Isolated From Patients Failing Antiretroviral Therapy in China

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**Summary:** Many AIDS patients in China who received free-of-charge antiretroviral therapeutics, including nucleoside reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs), showed significant life improvement. Increasing numbers of patients, however, are experiencing antiretroviral therapy failure due to the emergence of drug-resistant viruses. The aim of this study was to investigate the genotypic and phenotypic drug resistance patterns of HIV-1 subtype B' variants, which are prevalent in China, in order to rationally design more efficient anti-HIV-1 regimens for future treatment of AIDS patients. 13 out of 16 patients (81%) who were treated with two NRTIs (ddI, and d4T or AZT) and one NNRTI (NVP) exhibited high resistance to NVP, 8 of them with a >1,000 IC<sub>50</sub> fold increase. Five codons (101, 103, 108, 181, and 190) were involved in the NVP-resistant mutations, and K103N and Y181C mutations were predominant in these isolates. Fifteen isolates were resistant to at least one of the NRTIs, with high resistance to AZT (>10 IC<sub>50</sub> fold increase) and intermediate resistance to d4T or ddI (~4 IC<sub>50</sub> fold increase). More than 10 codons were involved in the NRTI-resistant mutation and located in two regions (M41-V75 and T215-K219) of the reverse transcriptase. Concordance between the genotype and phenotype patterns for both NRTIs and NNRTI were detected in a majority of the isolates, suggesting that phenotypic resistance is predictable from genotyping assays, which are faster and less expensive than phenotypic assay. Because NVP and AZT can induce high resistances in these patients, these two drugs should be replaced with others more effective NNRTIs and NRTIs.

**Key Words:** HIV-1, drug resistance, genotype, phenotype

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AIDS is a deadly disease caused by HIV infection; a great threat to worldwide public health.<sup>1</sup> China, with more than 1.3 billion people, also faces the threat of rapid spread of HIV/AIDS. About 650,000 people in China are living with HIV infection, including about 75,000 AIDS patients.<sup>2</sup>

With implementation of the “Four free, one care” policy (ie, free provision of antiviral drugs to HIV/AIDS patients, free anonymous HIV/AIDS tests, free services to prevent mother-to-child transmission, free tuition fees for AIDS orphans, and care for poor people living with HIV/AIDS and their families),<sup>3</sup> about 16,000 AIDS patients have been treated with free-of-charge antiretroviral (ARV) therapy in China, including nucleoside and nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Clinical applications of these anti-HIV drugs in various combinations, known as highly active antiretroviral therapy (HAART), have resulted in significant decreases of viral load and increases of CD4 T cells in treated patients.<sup>4</sup> However, the success of the HAART regimens is compromised by the emergence of drug-resistant HIV-1 variants caused by the presence of drug-selective pressures in ARV populations. HIV-1 genotypic and phenotypic resistance to NRTIs and NNRTIs has been well characterized in studies on internationally epidemic HIV-1 strains, especially subtype B.<sup>5</sup> However, little information is available about the RTI-resistant mutations in Chinese populations infected with HIV-1 subtype B' (Thailand subtype B) isolates. This study aims to analyze the genotype and phenotype patterns of drug-resistant HIV-1 subtype B' variants isolated from the Chinese patients failing ARV therapy. This information will help to rationally design more efficient anti-HIV-1 regimens for future treatment of HIV/AIDS patients in China.

## PATIENTS AND METHODS

### Study Population

Between March 2005 and November 2005, 16 HIV/AIDS patients who were treated with d4T + ddI + NVP (10 patients) or AZT + ddI + NVP (6 patients) for at least 6 months were investigated (see Table 1). All patients were infected with HIV-1 subtype B' and experienced ARV therapy failure (according to NIH ARV therapy failure criteria: (1) virologic failure, defined as repeated HIV RNA >400 copies/mL after 24 weeks in a treatment-naïve patient initiating therapy, or (2) immunologic failure, defined as failure to increase the CD4 cell count by 25 to 50 cells/mm<sup>3</sup>

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above the baseline count over the first year of therapy or a decrease to below the baseline CD4 cell count in therapy.<sup>6,7</sup> This study was approved by the Institutional Research Ethics Community, Chinese CDC, and all subjects signed informed consent forms before blood collection.

Blood viral loads were measured with COBAS AMPLICOR™ techniques and analyzer (Roche Diagnostics, Alameda, CA). This method has a lower limit of detection, ~500 copies/mL of HIV RNA in our laboratory. CD4<sup>+</sup> T lymphocytes in whole blood was quantitated by flow cytometry using reagents and equipment provided by Becton Dickinson Biosciences (San Jose, CA).

## Reagents

TZM-bl cells (JC57BL-13), obtained from the AIDS Research and Reference Reagent Program, NIAID, NIH, were derived from a HeLa cell clone. They contained integrated reporter genes for luciferase and *Escherichia coli* β-galactosidase under control of an HIV-1 LTR, which permits sensitive and accurate measurements of infection by most strains of HIV-1 R5 and X4 viruses, including primary HIV-1 isolates and molecularly cloned pseudoviruses.<sup>8,9</sup> HIV-1 III<sub>BA</sub>17 variant, HIV1617-1 variant, NRTIs (AZT, ddI and d4T), and NNRTI (NVP) were also obtained through the NIH AIDS Research and Reference Reagent Program.

## Isolation of HIV-1 From Peripheral Blood Mononuclear Cells (PBMCs) of HAART-Experienced Patients

PBMCs were isolated from blood by standard density-gradient centrifugation using Ficoll-Paque PLUS density-gradient medium (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). PBMCs isolated from HIV-1-infected individuals were cocultured with phytohemagglutinin (5 μg/mL) (Sigma Chemical Co., St. Louis, MO)-stimulated PBMCs from >2 HIV-1 sero-negative blood donors and cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2.9 mg/mL L-glutamine, and 100 IU recombinant IL-2 (Roche Diagnostics) as previously described.<sup>10</sup> Culture supernatants were collected once per week to measure p24 antigen levels using a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's recommendations (Bio-Merieux, Marcy-l'Etoile, France). Virus culture supernatants with p24 > 10 ng/mL were aliquoted and stored in liquid nitrogen until use.

## Nucleic Acid Extraction, Amplification, and Sequencing

Viral RNA was extracted from the isolated viruses using a QIAamp® Viral RNA Mini Kit (Qiagen Inc., Chatsworth, CA) according to the manufacturer's protocol, and stored at -70°C until use. For synthesis of cDNA, reverse transcription reaction was run with 5 μL RNA, 10 pmol downstream PCR primer RT21 (CTGTATTTCTGCTATTAAGTCTTTT GATGGG; HXB2 3509-3539), M-MuLV reverse transcriptase (New England BioLabs, Beijing, China), dNTPs (New England BioLabs, Beijing, China), and ribonuclease inhibitor (TaKaRa Biotechnology, Dalian, China) for 60 minutes at

45°C in a thermal cycler (GeneAmp® PCR system 9700, Applied Biosystems, Foster City, CA). A nested PCR strategy was employed to amplify the 1100-bp *pol* gene fragment encompassing the complete protease gene and the first 220 codons of the RT gene of HIV-1 RNA. Briefly, cDNA (10 μL) was used as a template, and the outer primers (MAW 26: TTggAAATgTggAAAggAaggAC; HXB2 2027-2050 and RT21) were used in the first round of PCR. The amplification was run at 94°C for 5 minutes, followed by 35 cycles at 94°C for 20 seconds, 55°C for 20 seconds, and 72°C for 2 minutes, and finally an extension of 7 minutes at 72°C. The first-round PCR product (5 μL) and the inner primers (PRO-1: CAgAgCCAACAgCCCCACCA; HXB2 2147-2166 and RT20: CTgCCAgTTCTAg CTCTgCTTC; HXB2 3441-3462) were used in the second round of PCR. The amplification was run at 94°C for 5 minutes, followed by 35 cycles at 94°C for 20 seconds, 55°C for 20 seconds, and 72°C for 1 minute, and finally an extension of 7 minutes at 72°C. The nested PCR product was purified using a QIAquick Gel Extraction Kit (Qiagen Inc.) was sequenced with the ABI 3100 DNA Sequencer (Applied Biosystems Inc.). The primers used for DNA sequencing were PRO-1, RT-20, RT-A (gTTgACTCAGATTggTTgCAC; HXB2 2519-2539), and RT-B (CCTAgTATAACAATgAgACAC; HXB2 2946-2967). For each PCR reaction, we used distilled water as blank control, RNA extracted from an HIV-free sample as negative control, and an RNA sample containing a well-known HIV sequence as positive control to monitor cross-contamination. In addition, the RNA extraction and PCR amplification were performed in two separate rooms to avoid aerosol contamination of the PCR amplicon.

## Analysis of Genotype Resistance

Sequences of the *pol* gene were compared to the consensus B reference sequence using HIVdb software (Stanford HIV Drug Resistance Database, <http://hivdb.stanford.edu>) to detect drug-resistance mutations and estimate the susceptibilities to d4T, AZT, ddI, and NVP, respectively. Genotypic susceptibility was classified into two groups: the resistant (R) group, including high-level resistance (H), intermediate-level resistance (I), and low-level resistance (L), and the sensitive (S) group, including sensitive and potential resistance (P).

## Virus Stock Titration

TZM-bl cells were resuspended in complete medium (DMEM medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2.9 mg/mL L-glutamine) and dispensed into a 96-well tissue culture plate at  $1 \times 10^4$  cells/well the day before infection. Virus stock was quickly thawed and diluted with the infection medium (addition of 30 μg/mL DEAE to the complete medium) and added to the cells in triplicate. After incubation for 48 hours at 37°C and 5% CO<sub>2</sub>, the medium was removed. Cells were fixed with phosphate-buffered saline containing 1% formaldehyde and 0.2% glutaraldehyde at room temperature and then stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, TaKaRa Shuzo Co., Shiga, Japan). Blue cells in each well were counted, and titration of the virus stock was determined

on the basis of single-round replication after 48 hours of incubation.

### Drug Susceptibility Assays Using TZM-bl Cells

Susceptibilities of HIV-1 isolates to AZT, d4T, ddI, and NVP were determined by using TZM-bl cells as previously described.<sup>9</sup> Briefly, TZM-bl cells were seeded ( $10^4$ /well) and cultured in a 96-well tissue culture plate overnight. They were exposed to diluted virus stock that produced 100–300 blue focus units (BFU). After incubation for 2 hours at 37°C and 5% CO<sub>2</sub>, the test drug was added in five serial dilutions (25, 5, 1, 0.1, and 0.01  $\mu$ mol/mL for ddI; 5, 1, 0.1, 0.01, and 0.001  $\mu$ mol/mL for AZT; 5, 1, 0.1, 0.01, and 0.001  $\mu$ mol/mL for d4T; and 5, 1, 0.1, 0.01, and 0.001  $\mu$ mol/mL for NVP, respectively). After further incubation at 37°C for 48 hours, luciferase activity was measured using a luciferase assay kit (Promega Corp) according to the manufacturer's instructions.

For the drug-susceptibility control, two HIV-1 strains, 020,200,842 and 020,200,222, isolated from antiretroviral-naïve patients who were paid blood donors in Anhui province and who were infected between 1993 and 1995, were used as the wild-type subtype B'. The two virus sequences of the *pol* region with similar nucleotide sequences as HIV-1 subtype B' were analyzed, and no mutation conferring resistance to the analyzed drug was found. Two HIV-1 variants, HIV-1 IIIBA17, harboring K103N and Y181C mutations and highly resistant to NNRTIs, and HIV1617-1, harboring NRTI mutations (K70G, 184V, 75I, 77L, 116Y, and 151M) and resistant to NRTIs (DDI, D4T, DDC, and AZT), were chosen as resistant controls.

### Phenotypic Analysis

Susceptibility or resistance of an HIV-1 isolate to a drug was determined by measuring the concentration of drug that inhibits 50% viral replication (IC<sub>50</sub>). A comparison of IC<sub>50</sub> values of viral isolate to those of wild-type reference strains provides a criterion to judge the level of resistance (fold increase in IC<sub>50</sub>). Results were reported as "sensitive" (S: <4-fold increase) and "resistant" (R), including intermediate resistance (I: 4- to 10-fold increase) and high-level resistance (H: >10-fold increase).<sup>11,12</sup>

### Statistical Methods and Analysis

Accordance between phenotype and genotype was calculated, and McNemar testing was applied to analyze the difference of two drug-resistance assays. We used 0.05 as the criterion for statistical significance, and exact testing was also applied due to the small sample size with StatXact Demo 7.0 (Cytel Inc., Cambridge, MA).

## RESULTS

### Characteristics of the Study Population

Sixteen AIDS patients (6 male and 10 female; average age, 39.1 years old) were recruited for this study. Ten patients received AZT + ddI + NVP, and 6 were treated with d4T + ddI + NVP for 6 months or longer. The average viral load was  $6.646 \times 10^5$  copies/mL (SD:  $1.06 \times 10^6$  copies/mL), and the average CD4 count was 143/ $\mu$ L (SD: 104/ $\mu$ L). Fourteen

patients exhibited ARV therapy failure, as shown by the viral load >500 copies/mL after 24 weeks in treatment-naïve patients initiating therapy. Two patients exhibited ARV therapy failure, as shown by the patients' CD4 cell counts not increasing by 25 to 50 cells/ $\mu$ L (Table 1).<sup>6,7</sup>

### Genotypic Analysis of the HIV-1 Subtype B' Variants Isolated From Patients Failing ARV Therapy

The amino acid sequences of the viral RTs were determined and compared to the consensus B reference sequence to identify RTI-resistance mutations and evaluate their susceptibilities to ddI, d4T, AZT, and NVP on the basis of sequence mutation sites. Fourteen out of 16 patients (8.7%) carried mutations conferring resistance to NVP, one of the NNRTIs. Triple mutations at positions K103 (7/16), Y181 (7/16), and G109 (5/16) in 5 patients and a single mutation at position K101 and V108 in 2 respective patients were found. All of the mutation sites were clustered in two regions (K101–V108 and Y181–G190) of HIV-1 RT (Fig. 1). Eight isolates had one or more genotypic resistance mutations to NRTIs, including M41L/M, A62V, K65R, D67N, T69A/P/S/T, K70K/N/R, L74V, V75T, T215F/Y, and K219Q (Table 2). Five isolates carried a T215 mutation. Three isolates have mutation at codons 41. Only one or two isolates carried mutations at codons 62, 65, 67, 69, 70, 74, 75, or 219. The mutation sites were clustered in the M41–V75 and T215–K219 regions of RT (Fig. 2).

### Phenotypic Analysis of the HIV-1 Subtype B' Variants Isolated From Patients Failing the Antiretroviral Therapy

The 16 virus stocks were tested for their phenotypic resistance using TZM-bl cells after analysis of genotypic susceptibility. Phenotypic data of clinical isolates were evaluated by the fold increase of IC<sub>50</sub> by comparing to the mean IC<sub>50</sub> of two controls' susceptibilities to drug (020,200,842 and 020,200,222). HIV1617-1 as a resistant control for NRTIs had phenotypic resistance to d4T (90-fold increase), ddI (67-fold increase), and AZT (>1000-fold increase). HIV-1 IIIBA17 variant, as a resistant control for

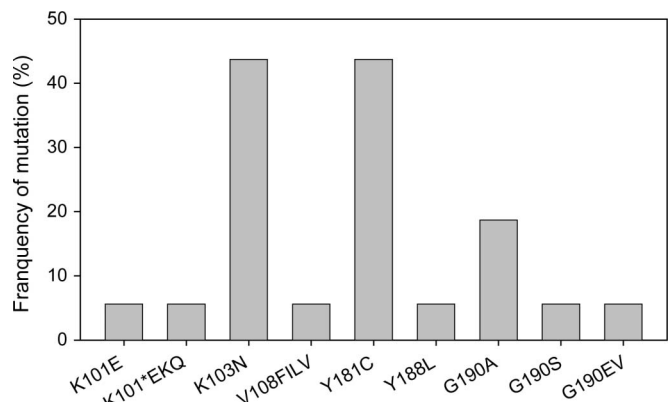


FIGURE 1. Prevalence of HIV-1 isolates with mutations for genotypic resistance to NNRTIs.

**TABLE 1.** Epidemiological and Clinical Information of the Participating HIV/AIDS Patients from Whom HIV-1 Subtype B' Viruses Were Isolated

Patient ID	Age (y)	Sex	Time Under HAART (mo)	HAART Regimen	Viral Load (copies/mL)	CD4 Counts (cells/ $\mu$ L)
239	41	F	12	D4T + DDI + NVP	$1.9 \times 10^5$	171
253	26	F	6	D4T + DDI + NVP	$1.6 \times 10^6$	63
062	50	F	18	D4T + DDI + NVP	$4 \times 10^6$	67
002	39	F	18	D4T + DDI + NVP	$2.9 \times 10^5$	317
083	48	M	18	D4T + DDI + NVP	$1.2 \times 10^6$	164
013	39	F	6	D4T + DDI + NVP	$5.1 \times 10^4$	287
040	34	M	18	D4T + DDI + NVP	$4.4 \times 10^5$	208
035	56	F	18	D4T + DDI + NVP	$5.8 \times 10^5$	74
100	49	F	6	D4T + DDI + NVP	$1.2 \times 10^6$	76
011	34	F	18	D4T + DDI + NVP	$8.176 \times 10^5$	348
788	33	M	9	AZT + DDI + NVP	$7.276 \times 10^4$	10
610	39	F	15	AZT + DDI + NVP	500	67
592	34	F	18	AZT + DDI + NVP	$5.010 \times 10^2$	79
604	37	M	12	AZT + DDI + NVP	$9.748 \times 10^3$	186
783	37	M	6	AZT + DDI + NVP	$1.810 \times 10^5$	144
580	29	M	12	AZT + DDI + NVP	500	22
Mean	39.06	M	12.75	AZT + DDI + NVP	$6.646 \times 10^5$	142.69

The value "<500" was treated as "500" for calculation convenience.

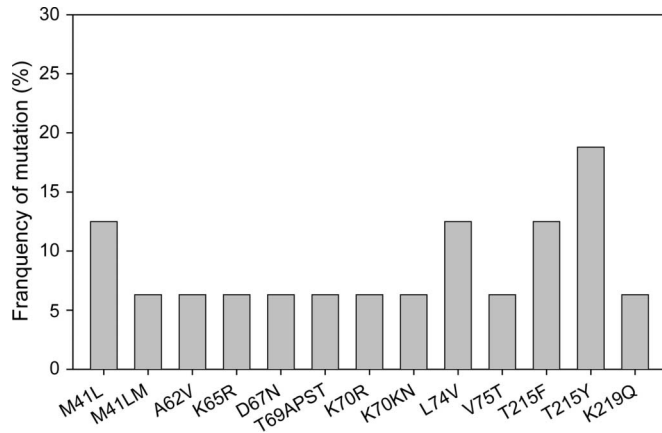
NNRTIs, had phenotypic resistance to NVP (>1000-fold increase). As shown in Table 2, 14 HIV-1 subtype B' variants with phenotypic resistance to at least one RTI were isolated from all patients who were treated with d4T + ddI + NVP or AZT + ddI + NVP. Among them, 14 isolates presented high-level resistance to NVP, most of which had >1000-fold

increase of IC<sub>50</sub>. Thirteen isolates were resistant to at least one of the NRTIs, and most of which showed low-level resistance to d4T or ddI with ~4-fold increase of IC<sub>50</sub>, while 7 of these isolates showed high-level resistance to AZT (>10-fold increase of IC<sub>50</sub>). Viruses resistant to AZT were isolated from 8 out of 10 patients who received the d4T + ddI + NVP

**TABLE 2.** Susceptibility to d4T, ddI, AZT, and NVP of Primary Isolates Containing Mutations Associated With Drug Resistance

Patient ID	Genotypic Resistance Mutations		Genotypic Resistance (Level)				Phenotypic Resistance (Level: Fold Increase of IC <sub>50</sub> )			
	NRTI	NNRTI	d4T	ddI	AZT	NVP	d4T	ddI	AZT	NVP
239	—	K103N, Y181C	S	S	S	R (H)	S (0.2)	R (I: 10)	S (<0.1)	R (H: >1000)
253	L74V	Y181C	S	R (I)	S	R (H)	S (3.6)	R (I: 10)	R (H: 57.4)	R (H: >1000)
062	A62V, V75T, K70KN,	Y188L	R (I)	S (P)	S (P)	R (H)	R (I: 4)	S (3.2)	R (I: 4)	R (H: >1000)
002	M41LM, T215Y	K103N, G190A	R (I)	R (I)	R (I)	R (H)	S (0.4)	S (1.8)	R (H: 179)	R (H: 449.6)
083	—	—	S	S	S	S	R (I: 7.3)	R (I: 4.2)	S (0.01)	R (H: 23.8)
013	—	—	S	S	S	S	S (2.7)	S (0.9)	R (I: 8.8)	S (0.6)
040	K65R	K103N, Y181C	R (L)	R (I)	S	R (H)	R (H: 18.6)	R (I: 10)	R (H: 89)	R (H: >1000)
035	M41L, L74V, T215F	K101E, Y181C, G190S	R (I)	R (H)	R (I)	R (H)	S (0.5)	R (I: 10)	R (H: 164.7)	R (H: >1000)
100	—	G190EV	S	S	S	R (H)	S (2.3)	R (I: 4.5)	R (I: 7.8)	R (H: 57.3)
011	—	K101*EKQ, Y181C, G190A	S	S	S	R (H)	S (1)	S (2.6)	R (H: 18.4)	R (H: 35.6)
788	—	—	S	S	S	S	S (1)	S (0.5)	S (I: 2.3)	S (1.9)
610	D67N, K70R, T215F, K219Q	K103N, Y181C	R (I)	R (L)	R (H)	R (H)	R (I: 6.4)	S (1.2)	R (H: 332.9)	R (H: >1000)
592	—	K103N	S	S	S	R (H)	S (0.3)	S (0.4)	S (0.4)	R (H: 22.5)
604	T215Y	K103N, Y181C	R (L)	R (L)	R (I)	R (H)	S (3.3)	S (1.4)	R (I: 6)	R (H: >1000)
783	T69APST	V108FILV	S	S	S	S (P)	S (3.7)	S (2.3)	S (I: 2.3)	S (<0.1)
580	M41L, T215Y	K103N, G190A	R (I)	R (I)	R (I)	R (H)	R (I: 10)	R (I: 4)	R (H: 912)	R (H: >1000)
Mean							4.08	4.19	111.54	536.96

Criteria for judging the genotypic and phenotypic resistance (R) and sensitivity (S) are described in Patients and Methods. Letters in boldface indicate discordance between genotype and phenotype patterns. To determine the phenotypic resistance of an HIV-1 isolate to a test drug, its IC<sub>50</sub> was measured and compared to two wild-type reference strains (020200842 and 020200222). Phenotypic data are expressed as the fold increase relative to the average IC<sub>50</sub> for the two reference strains. Dashes refer to absence of drug resistance-associated mutations.



**FIGURE 2.** Prevalence of HIV-1 isolates with mutations for genotypic resistance to NRTIs.

regimen, although no AZT was used. Similarly, the d4T-resistant variants were isolated from 2 out of 6 patients treated with the d4T + ddI + NVP regimen. These results suggest that one NRTI can induce cross-resistance to other NRTIs in ARV therapy-experienced patients.

### Relationship Between Genotype and Phenotype Patterns of the RTI-Resistant Isolates

By comparing the genotypic and phenotypic resistance profiles, we found that the viruses isolated from 15 out of 16 patients exhibited concordance between the genotype and phenotype resistance to NVP. However, ~1/4 to 1/3 of the isolates showed concordantly genotypic and phenotypic resistance to NRTIs while predominant variants showed both genotypic and phenotypic sensitivity to NRTIs. Both d4T and ddI induced genotypic and phenotypic resistance at intermediate level in some patients. AZT could induce high-level phenotypic resistance in 7 out of the 16 patients but induced high-level genotypic resistance only in 2 patients (Table 2). Statistical analysis showed there is no difference between genotypic and phenotypic susceptibility profiles of AZT, NVP, d4T, and ddI ( $P > 0.05$ ; Table 3). These data suggest that the phenotypic resistance of HIV-1 subtype B' variants induced by NRTIs (AZT, ddT, and d4T) and NNRTIs (NVP) is in concordance with their genotypic resistance.

### DISCUSSION

The paid blood donors were infected with HIV-1 in the central part of China, Henan province and its adjacent areas (such as Anhui province), in the 1990s, constituting the second major epidemic in China. Most of these patients were infected with HIV-1 subtype B'.<sup>13-15</sup> This strain is also known as Thai B, because it predominated initially with injection drug users in Thailand.<sup>16</sup> Since 2003, many of these patients have been treated with free-of-charge ARV therapeutics, including NRTIs and NNRTIs. More than 55% of the patients have shown significant clinical improvement in 3 months after antiretroviral therapy.<sup>4</sup> However, increasing numbers of

patients failing ARV therapy after 6 months of treatment. One major reason is the emergence of drug-resistant variants in these patients,<sup>4</sup> which mirrors reports from Western countries.<sup>5,17</sup> Our previous study focused on monitoring of HIV-1 drug resistance by genotypic assays.<sup>4</sup> The present study, for the first time in China, compared genotypic with phenotypic assays and determined a pattern of drug-resistant HIV-1 subtype B' variants isolated from patients failing ARV therapy and featured real-time analysis of the concordance between genotypic and phenotypic resistance profiles.

We found that all HIV-1 subtype B' variants isolated from 14 therapy-failure patients showed phenotypic resistance to at least one of the RTIs. Most isolates had a high-level resistance to NVP (an NNRTI) and AZT (an NRTI) (Table 2). About one-third of the isolates possessed phenotypic resistance to d4T and ddI (Table 3). The majority of isolates were found to have a drug-resistance profile that was in good concordance between genotypic and phenotypic resistance to RTIs in this study (Table 3). This is inconsistent with that reported by Sarmati and others,<sup>11</sup> in which a high rate of discordance was observed between genotypic and phenotypic drug-resistance profiles in HIV-1 variants isolated from patients who received double NRTIs (3TC + d4T or 3TC + AZT). This discrepancy could be attributed to a lack of NNRTI in their regimens. High concordance rates of genotypic and phenotypic resistance to NVP may be due to the fact that a single mutation at K103N or Y181C in RT could cause a high level of resistance to NNRTIs.<sup>18</sup> The high rate of concordance between genotype and phenotype resistance to RTIs in this study suggested that we could use genotype assays to monitor drug resistance instead of phenotype assay, because they are faster and more convenient than phenotype assays. Genotype assays could predict the phenotypic resistance of HIV-1 subtype B' variants in RTI-experienced patients. However, there is discordance between the genotypic and phenotypic assays in our study. The reasons are, firstly, that this genotypic assay only detects specific known sequence mutations; thus, interpretation of results is sometimes difficult in the presence of complex multiple mutations. Meanwhile, population-based sequencing methods in this study cannot detect minor resistant strains and cannot quantify the magnitude of resistance, causing discordance of two assays. Secondly, isolated viruses from

**TABLE 3.** Analysis of the Genotypic and Phenotypic Resistances of the HIV-1 Subtype B' Isolated From Patients Failing Antiretroviral Therapy

Resistance Pattern		Antiretroviral Drug			
Genotype	Phenotype	d4T	DDI	AZT	NVP
Resistance	Resistance	4	4	6	12
Sensitivity	Sensitivity	8	6	5	3
Resistance	Sensitivity	3	3	1	0
Sensitivity	Resistance	1	3	4	1
<i>P</i> value for McNemar testing		0.54	0.81	0.08	0.59

*P* values represent the statistical significance of differences between genotype and phenotype.  $P > 0.05$  shows that there is no difference between two drug-resistant tests.

patient samples required activated PBMCs to grow well. Therefore, the presence of viral quasi-species and PBMCs from different donor bloods could lead to significant interassay variations. So, phenotype assays using newly developed recombinant virus assays have better reproducibility, and accuracy should be further studied in designing antiretroviral therapy regimens for specific HIV-infected populations to show corresponding associations between the genotype and phenotype patterns.

There is significant cross-resistance induced by RTIs in ARV therapy patients in this study. Viruses isolated from most of the patients who received d4T + ddI + NVP showed high-level resistance to AZT, even in those who had never used AZT before. Some mutations (eg, Q151M) in RT induced by one RTI often caused cross-resistance to other RTIs.<sup>19</sup> However, only four RTIs (AZT, ddI, d4T, and NVP) were used in the drug-susceptibility assays of this study, and the result of the assay cannot totally reflect drug-resistant status of the patients because of the existence of cross-resistance; so, it is necessary to further analyze the genotype and phenotype pattern of other RTIs (ABC, 3TC, ddC, DLV, and EFV) in the future. Our previous studies have shown that cross-resistance can also be induced by NNRTIs.<sup>4</sup> As mentioned above, a single point mutation induced by one NNRTI can result in cross-resistance to other NNRTIs. This raises the serious concern that application of an inappropriate ARV regimen may lead to emergence of variants with multidrug resistance in therapy-experienced populations, resulting in a higher rate of ARV therapy failure. It is suggested that the current available ARV regimens in China should be revised and improved, especially for those who experience ARV therapy failure, on the basis of data generated by assessment of either HIV-1 genotype and/or phenotype patterns and that use of second-line ARV therapy may be urgently needed.<sup>20</sup>

In summary, this study suggested that, in China, a genotypic assay of AZT, ddI, d4T, and NVP could better predict the phenotypic outcome. The genotype may monitor AZT, ddI, d4T, and NVP resistance of HIV-1 subtype B' and further guide clinical ART in China. However, studies of genotype and phenotype drug resistance of other RTIs should also be carried out.

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