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HIV drug resistance in newly diagnosed adults in a rural prefecture of eastern China

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SUMMARY

Little is known about HIV drug resistance (HIVDR) in newly diagnosed HIV-infected adults in eastern China where the HIV epidemic is spreading predominantly through sexual contact. During 2008–2011, newly HIV-diagnosed adults in Taizhou prefecture, Zhejiang province in eastern China were examined for HIVDR by amplifying and sequencing the HIV-1 *pol* gene. Of 447 genotyped participants, 53·7% were infected with CRF01_AE, 20·1% with CRF07_BC, 12·5% with subtype B, and 11·6% with CRF08_BC. Most of the participants had one or more minor genetic mutations in the *pol* gene that are associated with HIVDR. Twelve (2·7%) participants met the standard guidelines of having low to high HIVDR, suggesting that the prevalence of HIVDR in newly HIV-diagnosed adults was low in the study area and current antiretroviral therapy (ART) regimens are likely to remain effective. However, given high frequency of minor HIVDR in HIV patients and the scaling up of ART programmes in China, larger HIVDR surveillance programmes are needed.

Key words: Antiretroviral therapy (ART), China, HIV drug resistance, transmission.

INTRODUCTION

Since 1996 combination antiretroviral therapy (cART) has offered HIV/AIDS-infected individuals the possibility of increased longevity, reduced HIV/AIDS-related complications, and improved quality of life [1–4]. At the end of 2011, 108 000 (14%) out of the estimated 780 000 people living with HIV/AIDS in China were on cART made available by the National Free Antiretroviral Treatment Programme (NFATP) [5, 6]. The number of HIV/AIDS-infected individuals on cART had significantly increased to 170 655 (22%) by the end of 2012. In the past decade, the NFATP has significantly reduced HIV/AIDS-related mortality and morbidity in China [3, 7, 8]. Nevertheless, amidst the rapid scaling up of cART there are concerns such as treatment non-compliance which might lead to incomplete suppression of HIV replication which in turn might lead to HIV drug resistance (HIVDR) [9, 10]. High prevalence of HIVDR would restrict therapy options, compromise the effect of current therapy regimens,

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and increase the risk of treatment failure. This is particularly problematic in resource-limited settings such as China where access to protease inhibitors (PIs) and new classes of antiretroviral drugs (ARVs) is still limited.

The World Health Organization (WHO) recommends surveillance of HIVDR in resource-limited settings where cART is being scaled up [11]. China first conducted surveillance of HIVDR in ART-naive HIV-infected individuals in 2004 [12]. By the end of 2012, the prevalence of HIVDR in ART-naive individuals ranged from 1·6% to 12·2% in China [12–24]. However, such surveillance data are not available for the most developed and populous areas in eastern China where the HIV epidemic is relatively new but rapidly spreading through unprotected heterosexual and homosexual behaviours.

To fill this significant gap, we conducted a molecular epidemiological survey during 2008–2011 to examine the prevalence of HIVDR in all newly diagnosed HIV-infected adults in a costal prefecture of Zhejiang province, eastern China. The knowledge gained from this study will be valuable for designing effective cART programmes for HIV/AIDS individuals in this region.

METHODS

Study site

This study was conducted in Taizhou prefecture of Zhejiang province, a coastal region in eastern China, which has a total of 5·9 million residents (National Bureau of Statistics of China, unpublished data). The first HIV case was reported in 1996. By the end of 2011, 886 HIV-infected individuals had been diagnosed and registered with the Chinese National Information System for AIDS Prevention and Control (CNISAPC). Of these, 69·3% were infected via heterosexual sex, 15·8% infected via homosexual sex, and 7·3% infected via injection drug use [25].

Study samples

A total of 671 adults were confirmed to be HIV-1 positive during the period of January 2008 to December 2011, of whom 513 (76·4%) had a minimum of 200 μl cryopreserved plasma sample at the time of their HIV diagnosis while naive to antiretroviral treatment (ART) and were eligible for HIV genotyping to determine HIVDR. The other 158 (23·6%) HIV-infected adults were not included for HIV genotyping and further analyses because of loss to follow-up after HIV diagnosis ($n=97$) or lack of plasma samples ($n=61$). The study was approved by the Institutional Review Board of Fudan University, China.

Ethical statement

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

RNA extraction and amplification

Plasma was separated from the whole blood within 2 h after collection and stored at −70 °C until use. Viral RNA was extracted from plasma samples using the High Pure Viral Nucleic Acid kit (Roche Inc., Germany) according to the manufacturer's instructions. The HIV-1 *pol* gene (protease 1–99 amino acids and part of reverse transcriptase 1–300 amino acids) was amplified by using an in-house nested reverse transcription–polymerase chain reaction (RT–PCR) method. The target sequence was amplified with TaKaRa One-Step RNA PCR kit (TaKaRa Biotechnology, China) using primers MAW26 (5′-TTGGAAATGTGGAAAG GAAGGAC-3′) and RT21 (5′-CTGTATTTCTGCTATTAAGTCTTTT-GATGGG-3′) in a 25 μl reaction. Cycling conditions were 50 °C for 30 min and 94 °C for 5 min in first-round RT–PCR, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min, and an extension at 72 °C for 10 min. The nested PCR was performed using the Takara Ex *Taq* PCR kit (TaKaRa Biotechnology), using primers PRO-1 (5′-CAGAG-CCAACAGCCCCACCA-3′) and RT20 (5′-CTGC-CAGTTC TAGCTCTGCTTC-3′) in a 50 μl reaction and the cycling conditions were 94 °C for 5 min in first-round RT–PCR, followed by 30 cycles at 94 °C for 30 s, 63 °C for 30 s, 72 °C 2·5 min, and an extension at 72 °C for 10 min.

Genotyping of HIVDR

Bi-directional sequences were obtained in the region of interest for all samples analysed in this study. For quality control, mutations presented as amino acid mixtures were identified only if the corresponding

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nucleotide mixture was present in the sequences of both DNA strands. The sequences of those amplified fragments were edited using the Lasergene software (SafeNet Co., USA). HIVDR and the susceptibility of the viruses to ART were interpreted with both the Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu) and the Surveillance Drug Resistance Mutations (SDRM) list recommended by WHO [26]. Furthermore, for each HIV-infected individual who was identified as having HIV genetic mutations that are not listed in the SDRM list, a cumulated mutation score (i.e. designated as the 'Total Scoring' in the Stanford HIVDR Database) was calculated by adding mutation scores (i.e. designated as 'Mutation Scoring' in the Stanford HIVDR Database) of all identified genetic mutations. To evaluate the possible impact of HIVDR transmission on the efficacy of future therapy with PIs, nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), we further analysed the HIV-1 *pol* gene sequences using the French National Agency on AIDS and Viral Hepatitis (ANRS) algorithm.

HIV-1 subtyping

The determination of HIV-1 subtypes was accomplished by submitting the obtained *pol* sequences including the entire PR gene and a part of the RT gene (1316 bp) to the HIV BLAST subtyping tool (http://www.hiv.lanl.gov) for phylogenetic analysis. The subsequent detailed phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA) software, version 5.1. The recommended HIV-1 subtype reference sequences available at the Los Alamos HIV sequences database (www.hiv.lanl.gov) were used. The phylogenetic reconstruction was performed using the neighbour-joining method based on the Kimura two-parameter distance model with 1000 bootstrapped datasets.

Statistical analysis

Statistical analysis was performed using SPSS software v. 17.0 (SPSS Inc., USA). Continuous variables were presented as mean±S.D. or median (interquartile range). Categorical variables were compared using χ^2 test or Fisher's exact test where appropriate. All tests were two-tailed and a P value <0·05 was considered as significant.

RESULTS

Characteristics of study participants

Of the 513 eligible study subjects, 447 (87·1%) were successfully genotyped with the HIV-1 *pol* gene. The failure of genotyping was primarily due to low plasma viral load and the inability to amplify viral RNA. Those who were genotyped and those who were non-genotyped were not significantly different in demographic characteristics (data not shown). Table 1 presents demographic characteristics of those who were genotyped, and these individuals were subjected to further analysis. The majority of them was male (73·2%), aged 18–40 years (66·7%), ethnic Han (94·0%), married (51·9%), and infected via heterosexual sex (69·8%).

HIV-1 subtypes

More than half (53·7%) of the genotyped study participants were infected with CRF01_AE, 20·1% with CRF07_BC, 12·5% with subtype B, 11·6% with CRF08_BC, 1·8% with subtype C and 0·2% with subtype A (Table 1). The distribution of HIV-1 subtypes did not vary by gender, age, and HIV report year, but did vary by HIV transmission route ($\chi^2 = 64{\cdot}90$, $P < 0{\cdot}001$). For the 312 heterosexually infected participants, 47·1% were infected with CRF01_AE, 20·5% with CRF07_BC, 14·1% with subtype B, 15·4% with CRF08_BC, 2·6% with subtype C and 0·3% with subtype A. For the 103 homosexually infected participants, 77·7% were infected with CRF01_AE, 12·6% with CRF07_BC, 8·7% with subtype B and 1·0% with CRF08_BC. For 21 infected injection drug users (IDUs), 28·6% were infected with CRF01_AE, 61·9% with CRF07_BC, 4·8% with subtype B, and 4·8% with CRF08_BC. For the eight participants who were infected via blood transfusion, four (50%) were infected with CRF01_AE, two (25%) with subtype B and two (25%) with CRF08_BC. All of the three individuals with transmission route unknown were infected with CRF01_AE.

Prevalence of HIVDR

Twelve patients were determined to have low to high HIVDR according to WHO SDRM criteria. The overall prevalence of HIVDR using WHO SDRM criteria in the study participants was 2·7% (12/447). Six (50%) of participants were only resistant to NRTIs, four (33·3%) were only resistant to NNRTIs, one

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Table 1. *Characteristics and prevalence of HIV drug resistance (HIVDR) among newly diagnosed ART-naive HIV-infected adults in Taizhou, 2008–2011*

Characteristics	Total [no. (%)]	HIVDR [no. (%)]	Wild type [no. (%)]	Prevalence of HIVDR (%)
Gender (χ^2=1·38, P=0·240)				
Male	327 (73·2)	7 (58·3)	320 (73·6)	2·1
Female	120 (26·8)	5 (41·7)	115 (26·4)	4·2
Age (years) (χ^2=0·43, P=0·807)				
18–40	298 (66·7)	7 (58·3)	291 (66·9)	2·3
41–60	114 (25·5)	4 (33·3)	110 (25·3)	3·5
61–86	35 (7·8)	1 (8·3)	34 (7·8)	2·9
Ethnicity (χ^2=0·11, P=0·735)				
Han	420 (94·0)	11 (91·7)	409 (94·0)	2·6
Others	27 (6·0)	1 (8·3)	26 (6·0)	3·7
Occupation (**χ^2=4·15, P=0·042**)				
Farmers	206 (46·1)	9 (75·0)	197 (45·3)	4·4
Others	241 (53·9)	3 (25·0)	238 (54·7)	1·2
Education (χ^2=0·03, P=0·983)				
Primary or lower	154 (34·5)	4 (33·3)	150 (34·5)	2·6
Middle school	191 (42·7)	5 (41·7)	186 (42·8)	2·6
High school or above	102 (22·8)	3 (25·0)	99 (22·8)	2·9
Marital status (χ^2=3·48, P=0·176)				
Single	141 (31·5)	3 (25·0)	138 (31·7)	2·1
Married	232 (51·9)	9 (75·0)	223 (51·3)	3·9
Divorced	74 (16·6)	0 (0·0)	74 (17·0)	0·0
HIV report year (χ^2=0·21, P=0·975)				
2008	61 (13·6)	2 (16·7)	59 (13·6)	3·3
2009	92 (20·6)	2 (16·7)	90 (20·7)	2·2
2010	157 (35·1)	4 (33·3)	153 (35·2)	2·5
2011	137 (30·6)	4 (33·3)	133 (30·6)	2·9
CD4 (cells/mm^3) (χ^2=2·14, P=0·545)				
0–200	85 (19·0)	1 (8·3)	84 (19·3)	1·2
201–350	131 (29·3)	5 (41·7)	126 (29·0)	3·8
351–500	154 (34·5)	5 (41·7)	149 (34·3)	3·2
⩾501	77 (17·2)	1 (8·3)	76 (16·5)	1·3
HIV transmission mode (χ^2=3·88, P=0·423)				
Heterosexual contact	312 (69·8)	9 (75·0)	303 (69·7)	2·9
Homosexual contact	103 (23·0)	2 (16·7)	101 (23·2)	1·9
Injection drug use	21 (4·7)	0 (0·0)	21 (4·8)	0·0
Blood transfusion	8 (1·8)	1 (8·3)	7 (1·6)	12·5
Unknown	3 (0·7)	0 (0·0)	3 (0·7)	0·0
HIV subtype (χ^2=4·70, P=0·454)				
CRF01_AE	240 (53·7)	4 (33·3)	236 (54·3)	1·7
CRF07_BC	90 (20·1)	2 (16·7)	88 (20·2)	2·2
CRF08_BC	52 (11·6)	3 (25·0)	49 (11·3)	5·8
B	56 (12·5)	3 (25·0)	53 (12·2)	5·4
C	8 (1·8)	0 (0·0)	8 (1·8)	0·0
A	1 (0·2)	0 (0·0)	1 (0·2)	0·0

(8·3%) was resistant to both PIs and NNRTIs, and one (8·3%) was resistant to all three classes of ART.

Table 1 presents the prevalence of HIVDR by demographic characteristics of the participants. The prevalence was significantly higher in farmers than other occupations combined (4·4% *vs.* 1·2%, χ^2=4·15, P=0·042). No other significant difference was observed.

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Predicted phenotypic HIVDR

According to the Stanford HIV Drug Resistance Database, 38 (8·5%) patients were predicted to be potentially resistant to ART, including the above 12 patients determined to have low to high HIVDR according to WHO SDRM criteria. Of these individuals, 25 (65·8%) were aged 21–40 years, 36 (94·7%) were ethnic Han, 23 (60·5%) were married, and 32 (84·2%) were infected with HIV via heterosexual sex. The median CD4 cell count of the 38 patients was 355 cells/mm^3.

Table 2 presents the detailed information of the 38 patients including their potential resistance to ART. Four (10·5%) harboured major PI mutations – M46I, V82A and L90M; 11 (28·9%) harboured minor PI mutations – A71 T, A71 V, L10F, L10I and L33F; 10 (26·3%) harboured NRTI-related mutations – A62 V, M184 V, T69N, T69S and T215S; and 23 (60·5%) harboured NNRTI-related mutations – G190A, K103N, V108I, V179D, V179E and Y188L, with V179D and V179E mutations occurring most frequently. As shown in Table 2, nevirapine (NVP; 21/38, 55·3%), efavirenz (EFV, 15/38, 39·5%) and rilpivirine (RPV, 8/38, 21·0%) were the drugs most likely to be less effective for treatment due to built-up HIVDR.

Minor amino acid substitutions associated with HIVDR

Most of study participants were found to have one or more minor genetic mutations or amino acid substitutions in the HIV *pol* gene that are associated with drug resistance (Table 3). Of them, 65·8% had I93L, 60·2% had M36I and 48·3% had L63P. Such amino acid substitutions occurred differentially by HIV subtypes (Table 3).

DISCUSSION

The present study, for the first time, examined HIVDR in newly diagnosed HIV-infected cases in Zhejiang province, eastern China where the HIV epidemic is rapidly spreading through sexual transmission. The study indicates low prevalence (<5%) of HIVDR in the study participants according to WHO classification [27], thus suggesting that current cART regimens are likely to remain effective in the study area. Such a low prevalence of HIVDR has also been observed in many regions in China [12–23]. The highest prevalence of HIVDR (12·2%) in ART-naive HIV patients was observed in Henan province where the HIV/AIDS epidemic started from commercial plasma donors in the early 1990s and large-scale cART was available earliest in the country [24]. A confluence of factors may contribute to such a relatively low prevalence of HIVDR in newly diagnosed or ART-naive HIV patients, for example, the relatively low coverage and short duration of NFATP as well as a success of first-line cART. It should be noted that the prevalence of HIVDR in the present study area was lower than an earlier study (2006–2007) of 145 treatment-naive HIV patients (7·6%) [15]. One possible explanation is that the 145 HIV patients in that study were from 22 provinces where the HIV epidemic as well as the coverage and duration of cART varied substantially.

The majority of HIVDR in our study are associated with reverse transcriptase inhibitors (RTIs). This finding reflects the fact that RTIs, usually two NRTIs + one NNRTI such as AZT + 3TC + EFV or AZT + 3TC + NVP, are the most widely used regimen in China as first-line cART. Previous reports demonstrate that HIV can develop a wide range of mutations resistant to RTI if viral suppression is incomplete [28–30]. On the other hand, some of the HIVDR in the study are associated with PIs, such as atazanavir (ATV), nelfinavir (NFV), fosamprenavir/r (FPV/r), indinavir/r (IDV/r) and saquinavir/r (SQV/r), which had not been introduced into the study area. These data suggest that HIV drug-resistant isolates from other countries may have 'slipped through' into eastern China or there are polymorphisms in subtypes prevalent in the region, which has implications for these individuals relying on the national ART programme. Moreover, six patients were identified to be resistant to multiple ART in the study. One of whom was found to have multiple HIV drug-resistant mutations (L90M, T215S, Y188L) that are associated with all the three classes of ART. The transmission of multiple HIVDR may have severe public health consequences and deserves more close scrutiny in the near future.

HIV mutations and amino acid substitutions associated with resistance to PIs and RTIs have been extensively characterized in subtype B [31–33], which is predominant in developed countries. However, such information is still relatively lacking in non-B subtypes, particularly in China. In this study, more than half of the study participants were infected with CRF01_AE, only a few (1·7%) of whom were infected with HIVDR. However, a relatively high prevalence

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Table 2. *HIV genotypic drug resistance mutations and predicted phenotypic drug resistance among newly diagnosed ART-naive HIV-infected adults in Taizhou, 2008–2011*

Sample ID*	Subtype	Gender	Age (yr)	CD4	Drug resistance mutations					Level of drug resistance			
					PI major	PI minor	NRTI	NNRTI	Other	Potential	Low	Intermediate	High
1	01AE	Female	21	459	M46I					ATV/r, FPV/r, IDV/r, LPV/r	**NFV**		
2	01AE	Male	48	320	M46I					ATV/r, FPV/r, IDV/r, LPV/r	**NFV**		
3	01AE	Male	59	275			M184 V			DDI	**ABC**		**3TC, FTC**
4	01AE	Male	24	290			T215S			ABC, DDI	**AZT, D4 T**		
5	07BC	Male	62	551	V82A	A71 V				FPV/r, SQV/r	**ATV/r, LPV/r**	**IDV/r, NFV**	
6	07BC	Female	48	10					E138G	ETR	**RPV**		
7	08BC	Female	37	242			A62 V, T69N			AZT, D4 T	**DDI**		
8	08BC	Female	36	410			A62 V, T69N			AZT, D4 T	**DDI**		
9	08BC	Male	46	231			A62 V, T69N			AZT, D4 T	**DDI**		
10	B	Male	27	449	L90M	L10I, A71 T	T215S	Y188L		LPV/r, ABC, DDI, ETR	**FPV/r, IDV/r, AZT, D4 T**	**ATV/r, SQV/r**	**NFV, EFV, NVP, RPV**
11	B	Male	24	437		A71 T	T215S			ABC, DDI	**AZT, D4 T**		
12	B	Female	38	426				K103N, G190A		ETR, RPV			**EFV, NVP**
13	01AE	Male	73	317		L10F				FPV/r, NFV			
14	01AE	Male	42	367		L10F				FPV/r, NFV			
15	01AE	Female	39	278		L33F		V179E		FPV/r ,EFV, NVP			
16	01AE	Male	35	428		L33F				FPV/r			
17	01AE	Female	24	730				V108I		NVP			
18	01AE	Male	45	330				V179D		EFV, ETR ,NVP, RPV			
19	01AE	Male	25	379				V179D		EFV, ETR, NVP, RPV			
20	01AE	Female	22	356				V179E		EFV, NVP			
21	01AE	Male	50	318				V179E		EFV, NVP			
22	01AE	Male	27	186				V179E		EFV, NVP			
23	01AE	Male	25	301				V179E		EFV, NVP			
24	01AE	Male	42	175				V179E		EFV, NVP			
25	07BC	Female	26	317		L10I, A71 T				NFV			
26	08BC	Male	40	233		A71 T	T69N	V179D		DDI, EFV, ETR, NVP, RPV			
27	08BC	Male	44	552			T69S	V179D		EFV, ETR, NVP, RPV			
28	08BC	Male	34	182			T69S	V179E		EFV, NVP			
29	08BC	Female	30	90				V179D		EFV, ETR, NVP, RPV			

Table 2 (*cont.*)

Sample ID*	Subtype	Gender	Age (yr)	CD4	Drug resistance mutations				Level of drug resistance			
					PI major	PI minor	NRTI	NNRTI	Other	Potential	Low	Intermediate	High
30	08BC	Male	40	31				V179D		EFV, ETR, NVP, RPV			
31	B	Male	44	470		A71 T		V179D		EFV, ETR, NVP, RPV			
32	B	Female	53	119		L10I, A71 T				NFV			
33	B	Male	40	237				V179D		EFV, ETR, NVP, RPV			
34	B	Male	38	380				V179E		EFV, NVP			
35	C	Female	22	874				V108I		NVP			
36	C	Female	29	595				V108I		NVP			
37	C	Female	23	513				V108I		NVP			
38	C	Male	27	650				V108I		NVP			

PIs, Protease inhibitors (ATV/r, atazanavir/r; FPV/r, fosamprenavir/r; IDV/r, indinavir/r; SQV/r, saquinavir/r; LPV/r, lopinavir/r); NRTIs, nucleoside reverse transcriptase inhibitors (DDI, didanosine; ABC, abacavir; 3TC, lamivudine; FTC, emtricitabine; AZT, zidovudine; D4 T, stavudine; TDF, tenofovir; NFV, nelfinavir); NNRTIs, non-nucleoside reverse transcriptase inhibitors (EFV, efavirenz; NVP, nevirapine; ETR, etravirine; RPV, rilpivirine).
* Samples nos. 1–12 were determined to have low to high level of phenotypic drug resistance, i.e. HIVDR.

of HIVDR was observed for CRF08_BC (3/52, 5·8%) and subtype B (3/56, 5·4%), although these two subtypes only accounted for 24% of the participants. CRF08_BC mainly conferred resistance to NRTIs, whereas subtype B mainly conferred resistance to NNRTIs.

Some minor amino acid substitutions associated with HIV drug resistance were identified in the study, which occurred differentially by HIV subtypes. Amino acid substitutions such as V77I and A71 V/T in protease were frequently found in subtype B. This observation was consistent with previous reports in China [17]. M36I accounted for 95·0% of the amino acid substitutions occurring in CRF 01_AE in this study. The improved viral replication offered by M36I might favour a more rapid spreading of non-B subtypes of HIV-1 [34–36]. I93L was primarily associated with non-A subtypes [36, 37], which was also observed in the present study. A62 V and T69N/S, both conferring resistance to NRTIs, were only found in CRF08_BC. Given the complexity of HIV subtypes circulating in this area and substantial differences in the profile of drug resistance mutations between the subtypes, it is of particular importance to implement HIV molecular surveillance programmes, especially HIVDR surveillance in newly HIV-infected individuals in the study area as well as other areas in China where multiple HIV genotypes are circulating. Knowledge gained from such programmes would be very useful for understanding HIV dynamics and designing optimal cART programmes in targeted programme sites.

Some limitations should be noted. First, all study participants were tested for HIVDR at the time of HIV diagnosis. However, due to low practice of HIV testing and delayed HIV diagnosis, many of the study participants might have been living with HIV for several years at the time of their HIV diagnosis. Thus, the possibility that the HIVDR identified in the study was not transmitted from other HIV-infected individuals with existing HIVDR but was developed by themselves after exposure to ART could not be ruled out, although such a possibility might be low given that the HIVDR was detected at the time of their HIV diagnosis and the availability of ART has been extremely limited for individuals without HIV infection in China. Second, given the relatively low prevalence of HIVDR in newly diagnosed HIV-infected individuals in the study area, the present study sample is not large enough to allow for more specific analysis of HIVDR. Future larger studies

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Table 3. *Minor amino acid substitutions associated with drug resistance in newly diagnosed ART-naive HIV-infected adults in Taizhou, 2008–2011*

Mutation	No. and proportion (%) of patients with amino acid substitutions								
	CRF01_AE ($n=240$)	CRF07_BC ($n=90$)	CRF08_BC ($n=52$)	B ($n=56$)	C ($n=8$)	A ($n=1$)	Total ($N=447$)	χ^2	P
Protease gene									
L10I/V/F	9 (3·8)	6 (6·7)		3 (5·4)		1 (100·0)	19 (4·3)	11·62	0·037
G16E	30 (12·5)						30 (6·7)	29·84	**0·000**
K20I/R	78 (32·5)	5 (5·6)		2 (3·6)			85 (19·0)	68·29	**0·000**
L33I/F	3 (1·3)						3 (0·7)	5·67	0·825
M36I	228 (95·0)	5 (5·6)	26 (50·0)	2 (3·6)	7 (87·5)	1 (100·0)	269 (60·2)	365·81	**0·000**
M46I	2 (0·8)						2 (0·4)	6·44	1·000
L63P	40 (16·7)	83 (92·2)	47 (90·4)	44 (78·6)	2 (25·0)		216 (48·3)	249·74	**0·000**
A71 T/V		12 (13·3)	1 (1·9)	16 (28·6)			29 (6·5)	63·36	**0·000**
V77I	13 (5·4)	24 (26·6)		51 (91·1)			88 (19·7)	198·91	**0·000**
V82A		1 (1·1)					1 (0·2)	10·61	0·463
L90M				1 (1·8)			1 (0·2)	11·56	0·262
I93L	108 (45·0)	80 (88·9)	52 (100·0)	46 (82·1)	8 (100·0)		294 (65·8)	120·59	**0·000**
Reverse transcriptase gene									
A62 V			3 (5·8)				3 (0·7)	14·94	**0·009**
T69N/S			6 (11·5)				6 (1·3)	24·84	**0·000**
V90I	1 (0·4)						1 (0·2)	8·64	1·000
K103N				1 (1·8)			1 (0·2)	11·56	0·262
V108I	1 (0·4)				4 (50·0)		5 (1·1)	31·35	**0·000**
E138G		1 (1·1)					1 (0·2)	10·61	0·463
V179D/E	8 (3·3)		5 (9·6)	3 (5·4)			16 (3·5)	10·86	0·052
M184 V	1 (0·4)						1 (0·2)	8·64	1·000
Y188I				1 (1·8)			1 (0·2)	11·56	0·262
G190A				1 (1·8)			1 (0·2)	11·56	0·262
T215S	1 (0·4)			2 (3·6)			3 (0·7)	9·31	0·192

especially large longitudinal cohort studies are warranted.

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DECLARATION OF INTEREST

None.

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