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RESEARCH PAPER

Genotypic characterization of human immunodeficiency virus type 1 isolated in Bali, Indonesia in 2016

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Abstract

Introduction: Bali is a famous tourism destination in Indonesia, and many international travelers from several foreign countries visit Bali. A large number of human immunodeficiency type 1 (HIV-1) infections was found; however, the genotypes of HIV-1 strains circulating in Bali has not yet been elucidated in detail. In addition, the information on HIV drug resistance (HIVDR) in Bali is limited.

Material and methods: HIV-1 *pol* gene encoding 31 proteases (PR gene) and 37 reverse transcriptase (RT gene) as well as 47 *gag* and 33 *env* genes were successfully amplified from DNA samples extracted from peripheral blood mononuclear cells samples of 51 antiretroviral therapy (ART)-experienced and 7 ART-naïve HIV-1-infected individuals residing in Gianyar and Denpasar, Bali. Genotyping analyses were then performed. The assessment of drug resistance mutations was based on the guidelines published by the International Antiviral Society-United States of America (IAS-USA).

Results: The major HIV-1 subtype detected in this study was CRF01_AE (94.5%). Recombinant viruses containing CRF01_AE and subtype B genes (5.5%) were also detected. Drug resistance-associated major mutations were detected in 8/31 (25.8%) of RT genes but not in PR genes in integrated proviral DNA derived from ART-experienced individuals. However, drug resistance-associated minor mutations were frequently detected in PR genes derived from most samples.

Conclusions: This study revealed that CRF01_AE was the predominant HIV-1 subtype in Bali, Indonesia. In addition, the emergence of drug resistance mutations was evident in proviral DNA derived from ART-experienced individuals, indicating that continuous surveillance is needed in order to monitor the emergence of HIVDR and new viral subtypes in Indonesia.

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Key words: HIV-1, Bali, Indonesia, subtype, antiretroviral therapy, drug resistance.

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Introduction

Human immunodeficiency virus type-1 (HIV-1) is the major causative agent of acquired immune deficiency syndrome (AIDS) [1]. The virus is characterized by extensive genetic heterogenicity. Due to this variability, HIV-1 is subdivided into 4 groups: M (major), O (outlying), N (new or non-M, non-O), and P (pending). Viruses in group M, which are responsible for the majority of infections in the worldwide HIV-1 pandemic [2, 3], are further classified into many subtypes or circulating recombinant forms (CRFs). Of these, subtypes A, B, C, D, and G, as well as CRF01_AE and CRF02_AG are the major subtypes and CRFs responsible for the worldwide pandemic. Intersubtype genetic variability has been reported among subtypes and CRFs; 15-20% of genetic variability is found in the *gag* gene and 25-35% in the *env* gene [1].

While subtype B of HIV-1 is the predominant subtype in the Americas, Europe, and Australia [4, 5], and is also present as a minority in Asia [6], a growing epidemic of non-B subtypes and CRFs has been reported in Africa [7] and Asia [8]. CRF01_AE is prevalent throughout Southeast Asia and responsible for more than 90% of infection cases in Indonesia [9]. Furthermore, several recombinant forms between CRF01_AE and subtype B, including CRF33_01B, have emerged in Indonesia [9-13]. Different subtypes and CRFs are considered to show different rates of disease progression, immune responses, responses to antiretroviral therapy (ART), and the development of drug resistance [14]. Therefore, it is important to monitor the global prevalence of subtypes and CRFs for the prevention and control of HIV-1 as well as vaccine development.

Antiretroviral therapy (ART) has markedly reduced the transmission, morbidity, and mortality associated with HIV. In 2013, number of HIV-infected individuals in Indonesia with $CD4^+$ T-cell count less than 500 cells/mm^3 were estimated to be 510,000, and these individuals were eligible for ART according to the World Health Organization (WHO) criteria. The Indonesian national ART program enabled 40% of these individuals to access ART in 2011 [15]. The first-line regimen of ART recommended in Indonesia is a combination of two nucleoside reverse-transcriptase inhibitors (NRTIs) and a non-nucleoside reverse-transcriptase inhibitor (NNRTI). Lamivudine (3TC), zidovudine (AZT), tenofovir (TDF), nevirapine (NVP), and efavirenz (EFV) are commonly used. In patients with virological failure and/or adverse effects to the first-line regimen, ritonavir-boosted protease inhibitors in combination with two NRTIs are recommended as the second-line regimen. Other drugs, including didanosine (ddI), etravirine (ETR), and rilpivirine (RPV), are uncommon in the country. Although ART is successful in Indonesia, the emergence of drug resistance has been reported among treatment failure cases as well as ART-naïve individuals [16-19].

Bali is a famous tourism destination in Indonesia, and many international travelers from several foreign countries visit Bali. It is assumed that some of these travelers may be HIV-infected. According to Indonesian Ministry of Health, Bali has the fourth largest number of HIV cases in Indonesia. Previous studies revealed a large number of HIV infection in Bali [20, 21]. However, the genotypes of HIV-1 strains circulating in Bali has not yet been elucidated in detail. In addition, the information on HIVDR in Bali is limited. In order to clarify the circulating HIV-1 subtypes and monitor the emergence of HIVDR in Bali, a genotypic study was conducted on viral genomic fragments derived from peripheral blood samples of ART-experienced or ART-naïve HIV-1-infected individuals residing in Gianyar and Denpasar, Bali.

Material and methods

Ethics statement

This study was conducted with approvals from the Medical Research Ethics Committees of the Faculty of Medicine of Udayana University, Airlangga University, and Kobe University Graduate School of Medicine. All study participants were enrolled after providing written informed consents.

Study site, patient recruitment, and sample collection

Fifty-eight HIV-infected individuals, consisting of 51 ART-experienced and 7 ART-naïve individuals, were recruited from the Sinta HIV Clinic of Sanjiwani Hospital in Gianyar regency and from Puskesmas Denpasar Selatan II at Denpasar municipality in Bali, Indonesia. Eight milliliters whole blood samples were collected from study participants between January and March 2016. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation for 10 minutes at 1,800 rpm using the BD Vacutainer® CPT™ Cell Preparation Tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). DNA was then extracted from PBMCs using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Demographic, clinical, and hematological data as well as disease severity according to the WHO guidelines of study participants were retrieved from the medical records.

HIV-1 genotypic analysis

The HIV-1 *pol* gene encoding protease (PR gene) and reverse transcriptase (RT gene) as well as *gag* and *env* genes were amplified by nested polymerase chain reaction (PCR) using Gotaq green master mix (Promega, Madison, WI, USA). The primer sets used were as follows. In order to amplify the viral PR gene, the primers DRPRO5, 5′-AGACAGGYTAATTTTTTAGGGA-3′ ((corresponding to nucleotides (nt) 2074-2095 of an HIV-1 reference strain, HXB2 (GenBank accession no. K03455)) and DRPRO2L, 5′-TATGGATTTTCAGGCCCAATTTTTGA-3′ (nt 2716 to 2691) were used for the first PCR, and the primers DRPRO1M, 5′-AGAGCCAACAGCCCCACCAG-3′ (nt 2148 to 2167), and DRPRO6, 5′-ACTTTTGGGCCATCCATTCC-3′ (nt 2611 to 2592) were used for nested PCR. In order to amplify the viral RT gene, RT1L, 5′-ATGATAGGGGGAATTGGAGGTTT-3′ (nt 2388

to 2410) and DRRT4L, 5′-TACTTCTGTTAGTGCTTTG-GTTCC-3′ (nt 4402 to 4380) were used for first PCR, and RT7L, 5′-GACCTACACCTGTCAACATAATTGG-3′ (nt 2485 to 2509) and DRRT6L, 5′-TAATCCCTGCATAAATCT-GACTTGC-3′ (nt 4309 to 4285) were used for nested PCR. In order to amplify the *gag* gene encoding Gag p24, the primers for first PCR were H1G777, 5'-TCACCTAGAACTTT-GAATGCATGGG-3' (nt 1231 to 1255) and H1P202, 5'-CTA-ATACTGTATCATCTGCTCCTGT-3' (nt 2352 to 2325), while the primers used for nested PCR were H1Gag1584, 5'-AAAGATGGATAATCCTGGG-3' (nt 1577 to 1595) and G17, 5'-TCCACATTTCCAACAGCCCTTTTT-3' (nt 2040 to 2017). In order to amplify the C2-V3 regions of *env* gene, the primers used for first PCR were M5, 5'-CCAATTCCCAT-ACATTATTGTGCCCCAGCTGG-3' (nt 6858 to 6889) and M10, 5'-CCAATTGTCCCTCATATCTCCTCCTCCAGG-3' (nt 7661 to 7632), while the primers used for nested PCR were M3, 5'-GTCAGCACAGTACAATGIACACATGG-3' (nt 6948 to 6973) and M8, 5'-TCCTTGGATGGGAGGGG-CATACATTGC-3' (nt 7547 to 7521). The PCR product amplified at the end-point dilution of DNA templates were subjected to a sequencing analysis in order to examine the genomic fragment of the major viral population in a sample.

The BigDye Terminator version 3.1 Cycle Sequencing kit and ABI PRISM3500Xl genetic analyzer (Applied Biosystems, Foster City, CA, USA) were used in the sequencing analysis of amplified HIV-1 *gag*, *pol*, and *env* genes. Data were assembled using Genetyx version 10 software (Genetyx, Tokyo, Japan). HIV-1 subtyping was conducted using the Recombinant Identification Program (RIP) available on the HIV sequence database website (www.hiv.lanl.gov) and jumping profile Hidden Markov Model (jpHMM)-HIV (http://jphmm.gobics.de/submission_hiv). Neighbor-joining trees with a Kimura two-parameter model were constructed using MEGA6.2 software with bootstrap values (1,000 replicates) for relevant nodes being reported on a representative tree. When a discrepancy was found in the subtyping of a sample among the *gag*, PR, RT, and *env* genes, it was defined as a unique recombinant form of more than two HIV-1 subtypes and CRFs. The appearance of drug resistant mutations in the 31 PR and 37 RT genes was studied according to the International Antiviral Society-United States of America (IAS-USA) panel [22]. The nucleotide sequences of the PR, RT, *gag*, and *env* genes have been registered in the GenBank database under accession numbers KY985644-KY985676 (*env* genes), KY985677-KY985723 (*gag* genes), KY985724-KY985760 (RT genes), and KY985761-KY985791 (PR genes).

Results

Demographic and clinical information of study participants

The demographic information of 58 study participants is shown in Table 1. The mean age of study participants was 36.5 (SD ± 11.66) years old. Study participants had a median CD4 cell count of 52.50 cells/mm^3, with an interquartile range (IQR) of 1-480 cells/mm^3. Most ethnic group were from Bali (94.8%). The main route of infection was heterosexual intercourse (87.9%). Six individuals were ART-naïve (10.3%), while 52 individuals had been on ART (89.7%). The fixed combination of AZT/3TC/NVP (17.3%), AZT/3TC/EFV (10.3%), TDF/3TC/EFV (44.8%), or TDF/3TC/NVP (17.3%) was used in the treatment of individuals on ART. The mean duration of ART usage was 26.68 (SD ± 17.84) months. Fourteen individuals were in the non-AIDS stage (24.1%), while 44 individuals were in the AIDS stage (75.9%). Fifty-one individuals had developed opportunistic infections (87.9%).

Table 1. Demographic characteristics of study participants

Characteristics	Value (*n* = 58)
Age, mean, years (SD)	36.54 (11.66)
CD4, pre-ART, median (IQR)	52.50 (1-480)
Gender, *n* (%)	
Male	37 (63.8%)
Female	21 (36.2%)
Marital status, *n* (%)	
Married	48 (82.8%)
Single (divorced/widowed)	10 (17.2%)
Ethnic group, *n* (%)	
Bali	55 (94.8%)
Java	2 (3.5%)
East Nusa Tenggara	1 (1.7%)
Transmission risk category, *n* (%)	
Heterosexual	51 (87.9%)
Injecting drug use	1 (1.7%)
Men who have sex with men	4 (6.9%)
Mother-to-child transmission	2 (3.4%)
Types of ART used, *n* (%)	
AZT+3TC+NVP	10 (17.3%)
AZT+3TC+EFV	6 (10.3%)
TDF+3TC+EFV	26 (44.8%)
TDF+3TC+NVP	10 (17.3%)
Naïve	6 (10.3%)
Duration of ART, *n* (%)	
< 1 year	15 (25.9%)
1-3 years	23 (39.7%)
> 3 years	14 (24.1%)
HIV stage	
Non-AIDS	14 (24.1%)
AIDS	44 (75.9%)
History of OI, *n* (%)	
No	7 (12.1%)
Yes	51 (87.9%)

Table 2. HIV-1 subtyping

Sample ID	Subtype/ CRF assignment	Subtyping			
		PR gene	RT gene	*gag* gene	*env* gene
H01	CRF01_AE	NA*	CRF01_AE	CRF01_AE	NA
H02	CRF01_AE	NA	CRF01_AE	NA	NA
H03	CRF01_AE	NA	NA	CRF01_AE	CRF01_AE
H04	NA	NA	NA	NA	NA
H05	CRF01_AE	NA	NA	CRF01_AE	NA
H06	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
H07	CRF01_AE	NA	CRF01_AE	CRF01_AE	NA
H08	CRF01_AE	CRF01_AE	NA	CRF01_AE	CRF01_AE
H09	NA	NA	NA	NA	NA
H010	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H011	CRF01_AE/B**	NA	NA	CRF01_AE	B
H012	NA	NA	NA	NA	NA
H013	CRF01_AE	NA	NA	CRF01_AE	NA
H014	CRF01_AE	CRF01_AE	NA	CRF01_AE	NA
H015	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H016	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H017	CRF01_AE	NA	NA	CRF01_AE	NA
H018	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
H019	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H020	CRF01_AE	CRF01_AE	NA	NA	CRF01_AE
H021	CRF01_AE	NA	NA	CRF01_AE	NA
H022	CRF01_AE	NA	CRF01_AE	NA	NA
H023	CRF01_AE	CRF01_AE	CRF01_AE	NA	CRF01_AE
H024	CRF01_AE	CRF01_AE	NA	CRF01_AE	CRF01_AE
H025	CRF01_AE	NA	CRF01_AE	CRF01_AE	NA
H026	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H027	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
H028	CRF01_AE	CRF01_AE	NA	NA	NA
H029	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H030	CRF01_AE/B	NA	CRF01_AE	B	CRF01_AE
H031	CRF01_AE	NA	NA	CRF01_AE	CRF01_AE
H032	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H033	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H034	CRF01_AE	NA	NA	CRF01_AE	CRF01_AE
H035	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H036	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H037	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H038	CRF01_AE	NA	CRF01_AE	NA	NA
H039	CRF01_AE	NA	CRF01_AE	CRF01_AE	CRF01_AE
H040	CRF01_AE	CRF01_AE	NA	CRF01_AE	CRF01_AE
H041	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H042	CRF01_AE	CRF01_AE	CRF01_AE	NA	NA
H043	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 2. Cont.

Sample ID	Subtype/ CRF assignment	Subtyping			
		PR gene	RT gene	*gag* gene	*env* gene
H044	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H045	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H046	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
H047	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H048	CRF01_AE	CRF01_AE	NA	NA	NA
H049	CRF01_AE	CRF01_AE	NA	CRF01_AE	CRF01_AE
H050	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H051	CRF01_AE/B	B	CRF01_AE	CRF01_AE	NA
H052	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
H053	CRF01_AE	CRF01_AE	NA	CRF01_AE	CRF01_AE
H054	CRF01_AE	CRF01_AE	NA	CRF01_AE	CRF01_AE
H055	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
H056	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
H057	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	NA
H058	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

**Not available due to the failure of PCR*

***Recombinant form of HIV-1 containing viral gene fragments of CRF01_AE and subtype B*

Table 3. Distribution of HIV-1 subtypes by gender

HIV-1 subtype	HIV/AIDS patients		
	Total, *N* = 55	Male, *n* = 35	Female, *n* = 20
CRF01_AE	52 (94.5%)	34 (97.1%)	18 (90%)
CRF01_AE/B*	3 (5.5%)	1 (2.9%)	2 (10%)

**Recombinant viruses between CRF01_AE and subtype B*

HIV-1 subtyping

The sequencing data of 31 PR genes (297 bp; nt 2253 to 2,549), 37 RT genes (762-bp; nt 2,550 to 3,311), the partial fragments of 47 *gag* genes encoding Gag p24 (382-bp; nt 1,627 to 2,008), and the partial fragments of 33 *env* genes spanning the C2-V3 region (389-bp; nt 7,020 to 7,408) were obtained from 55 blood samples, while no sequencing data was available from 3 samples (Table 2). According to RIP and jpHMM-HIV results as well as the phylogenetic tree analysis, CRF01_AE was the dominant subtype in Bali (94.5%) (Table 3). In addition, recombinant viruses containing CRF01_AE and subtype B gene fragments (5.5%) were detected (Table 3). Viral subtyping by RIP, jpHMM-HIV, and phylogenetic trees showed consistent results (data not shown).

Appearance of drug resistance-associated mutations

The appearance of drug resistance-associated mutations was evaluated on successfully sequenced 31 RT and 26 PR genes derived from ART-experienced individuals. Drug resistance-associated major mutations against NRTIs and NNRTIs were found in 8/31 RT genes (25.8%) in integrated proviral DNA derived from ART-experienced individuals (Table 4). In particular, samples H033 and H056 contained several major mutations, including thymidine analogue-associated mutations (TAMs), which confer multi NRTI resistance (Table 4). In PR genes, no drug resistance-associated major mutations were detected (data not shown). However, minor mutations, M36I ((amino acid substitution from methionin (M) to isoleucine (I) at position 36 in PR gene)) (100%), H69K (100%), and L89M (88.5%) were commonly detected in the PR gene (Table 4) [21]. These mutations confer resistance to ritonavir-boosted indinavir (IDV/r), atazanavir (ATV/r), fosamprenavir (FPV/r), lopinavir (LPV/r), tipranavir (TPV/r), and nelfinavir (NFV).

Discussion

HIV-1 genetic diversity is caused by viral mechanisms that elude immune control and drug pressure. The existence of distinct subtypes and CRFs reflects the presence of a dy-

Table 4. Antiretroviral drug resistance-associated mutations in 8 individuals on ART

Sample ID	ART regimen and duration	Drug resistance mutations*		
		NRTI	NNRTI	PI
H010	FDC, more than 3 years	**M184V**	**Y181C** **Y188L**	NA**
H018	FDC, 2 years	**–*** **	E138G	M36I H69K V82I L89M I93L
H030	FDC, 18 months	–	V90I	NA
H033	3TC+AZT+EFV, 43 months	**M41L** **D67N** **K70R** **M184V** **L210W** **T215Y** **K219E**	A98G **K103N** **V108I**	NA
H035	EFV TRIPLE, 2 years	**M184V**	**K103N** **Y188L**	G16E K20R M36I H69K L89M
H043	FDC	**M184V**	**K103N**	G16E K20R M36I H69K L89M
H055	3TC+AZT+NVP, 18 months	**–**	**M30L**	K20R M36I H69K V77I L89M
H056	TDF+3TC+EFV, 11 months	**D67N** **K70R** **K219E**	**Y181C**	G16E M36I H69K L89M

**The assessment of drug resistance mutations was based on the guidelines published by the International Antiviral Society-United States of America (IAS-USA). Drug resistance-associated major mutations are shown in bold*

***Not available due to the failure of PCR*

****Not detected*

namic genetic evolutionary process of the virus. The most prominent HIV-1 subtype in Bali found in the present study was CRF01_AE, which is widely prevalent throughout Southeast Asian countries including Indonesia [8, 9, 17]. Previous HIV-1 genotyping studies in Indonesia reported similar findings. Roselinda and Jekti [23] found that CRF01_AE viruses circulated in 7 provinces in Indonesia as the predominant subtype, followed by subtype B and recombinant viruses containing subtypes A and G gene fragments, which were not found in the present study. CRF01_AE was found in 80% of the samples in North Sumatra province and in East Java, while it was only detected in a quarter of samples in the Papua provinces [23, 24]. A study in Malaysia, a neighbor country of Indonesia, reported similar findings for the HIV-1 subtype distribution in that CRF01_AE was the most dominant strain and other subtypes were identified as minorities [25]. In contrast to this study, subtype B (41.9%) was the major subtype prevalent in Sorong, West Papua, Indonesia [11]. Differences in the distribution of the HIV-1 subtype in Indonesia may be caused by many factors such as different high-risk behaviors for HIV-1 infection, genetic and demographic characteristic of individuals residing in each region, geographic, economic, and social factors. Diverse high-risk behaviors sustain HIV-1 transmission in different regions of the world, and multiple transmission routes may be involved in spreading the HIV-1 epidemic within the same

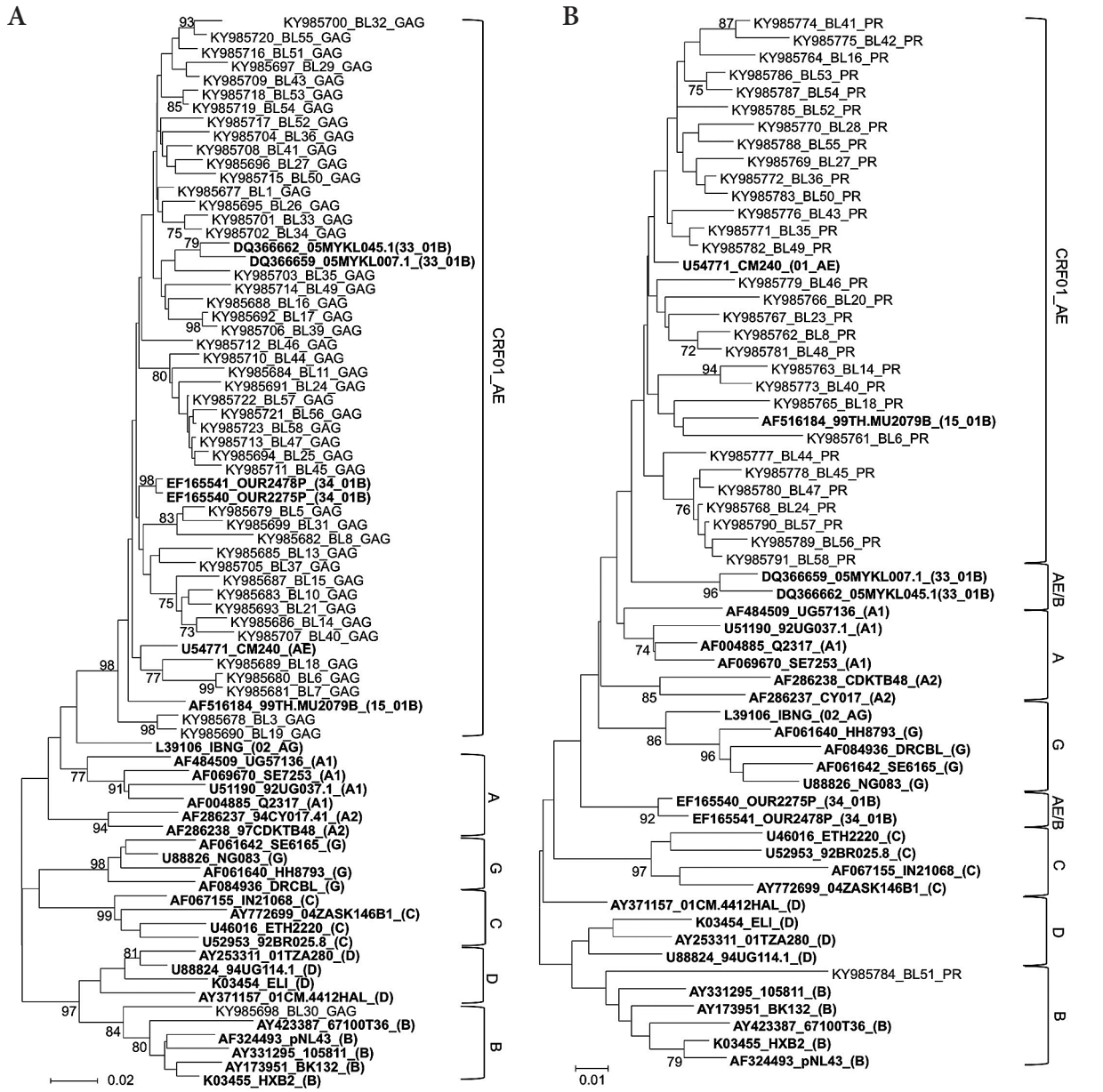

Fig. 1. Phylogenetic analysis of HIV-1 PR, RT, *gag*, and *env* gene sequences. Phylogenetic trees were generated for the newly sequenced HIV-1 *gag* **(A)**, PR **(B)**, RT **(C)**, and *env* genes **(D)** together with the corresponding viral genes of reference HIV-1 strains representing subtype A1 (A1), subtype A2 (A2), subtype B (B), subtype C (C), subtype D (D), subtype G (G), CRF01_AE (01_AE), CRF02_AG (02_AG), CRF15_01B (15_01B), CRF33_01B (33_01B), and CRF34_01B (34_01B). The reference strains of the HIV-1 subtype are shown in bold. Sequence codes are presented as the GenBank accession number, patient ID or name of the reference strain, and the subtype or CRF of the reference strain (shown in parentheses) in order. Bootstrap values were shown when values were > 70. CRF – circulating recombinant form

region [1]. As an RNA virus, HIV-1 replication is characterized by high mutation rates, leading to diversification of its strains. Intersubtype recombination contributes to HIV-1 sequence diversity, which is generated by the co-circulation of two or more different subtypes and/or CRFs in high-risk populations [26]. Other recombinant forms, such as recombinant viruses between CRF01_AE and subtype B (5.5%) were also detected in the present study. The prevalence of recombinant forms of HIV-1 in Indonesia was reported to be low. Namely, the low prevalence of recombinants between CRF01_AE and subtype B was similar to previous findings in Surabaya (3.0%) and in Sorong (2.3%) [11, 17]. Subtype B is the most prevalent HIV-1 subtype in North America, Western Europe, and Australia; however, Sides *et al.* found that non-B subtypes

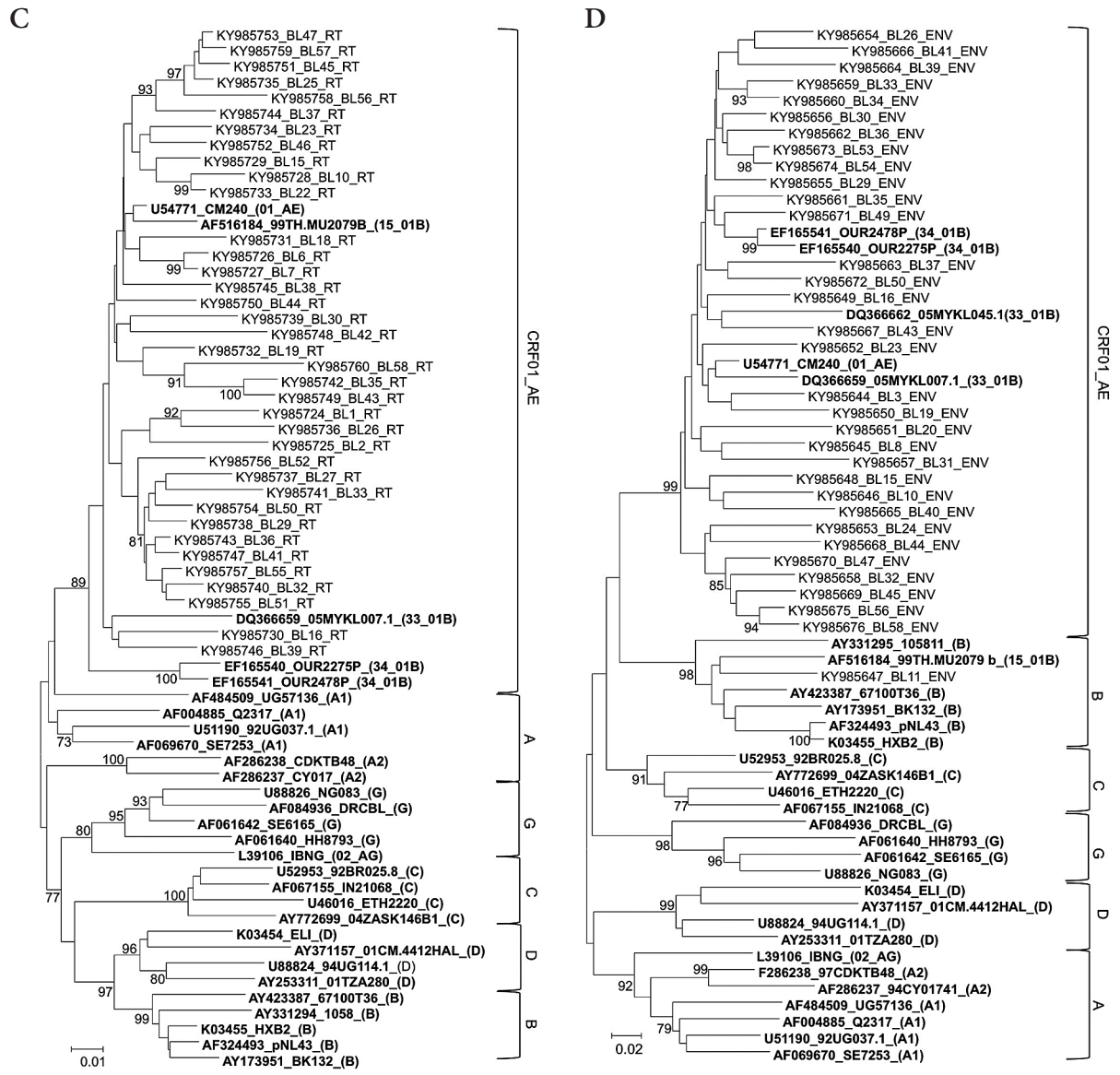

Fig. 1. Cont.

were prevalent as the major subtypes among African-born HIV-1-infected individuals in Minnesota, USA [27], suggesting that migration may have affected the subtype distribution in the world. Furthermore, the epidemic of a minor lineage of HIV-1 subtype B (termed subtype B') occurred in Asia [6]. Therefore, it is important to continuously monitor the prevalence of HIV-1 subtypes and CRFs worldwide.

Genetic variability was found in viral PR and RT genes coding the viral enzymes targeted by antiretroviral drugs. If a drug resistance-associated mutation appears as a result of a genetic polymorphism, it may be selected further by drug-selective pressure, and markedly influence therapeutic outcome [1]. In the present study, drug resistance-associated major mutations were detected in RT genes derived from 8 ART-experienced individuals. In particular, several mutations were detected in 2 individuals treated with the combination of 2 NRTIs and 1 NNRTI. Patient H033 had been treated with 3TC/AZT/EFV for 43 months. Several TAMs (M41L, D67N, K70R, and M184V) and mutations against NNRTIs (K103N, V108I) was found in the viral RT gene derived from the patient. Based on clinical data, this patient had not developed any opportunistic infections, ART side effect, or ART failure (data not shown). The mutations, M184V and K103N are commonly detected as multi-drug resistance mutations leading to the virological failure of a first-line NRTIs- and NNRTI-based regimens [16, 28]. The appearance pattern of these drug resistance mutations was similar with those in previous studies in Indonesia, Malaysia, Taiwan, Turkey, and USA [17, 18-31]. M184V is a common mutation in treated populations; however, it is very rare among

ART-naïve individuals. This may be due to that M184V deteriorates viral fitness in the absence of ART. In present study, drug resistance-associated major mutations were not found in RT genes derived from ART-naïve patients, while several TAMs (M41L, D67N, K70R, L210W, T215Y, K219E) were detected in ART-naïve individuals as transmitted drug resistance (TDR) in a previous study conducted in Surabaya, Indonesia [19]. Due to a limited sample size, the emergence of TDR among ART-naïve individuals cannot be evaluated in this study. Since drug resistance-associated mutations have frequently been detected among treatment-failure patients in Indonesia, there may be a risk of TDR among newly infected individuals. This study needs to be continued with a larger sample size, improved sampling quality, and direct sequencing in order to obtain the best TDR estimation in HIV-1-infected individuals in Indonesia. In addition, several minor mutations were found in PR genes derived from most study participants. Although these mutations may also affect clinical manifestations, this was not evaluated in present study. Minor mutations have been detected in PR genes of non-B subtypes, including CRF01_AE, as natural polymorphisms [14]. Drug resistance-associated minor mutations in PR genes have also been reported in other studies in Indonesia [11, 17, 18].

Conclusions

CRF01_AE is the dominant HIV-1 strain prevalent in Bali. The emergence of drug resistance-associated major mutations was evident among patients on ART. The detection of drug resistance-associated mutations in ART-naïve and ART experienced samples, and the combining of data with clinical, immunological, and virological data of HIV-1-infected individuals are needed in order to improve the clinical outcomes of ART in Indonesia.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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