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Genotypic characterization of human immunodeficiency virus type 1 isolated from ART-experienced individuals in Buleleng Regency, Bali, Indonesia

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Running head: HIV-1 epidemiology in Buleleng, Bali, Indonesia

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Abstract

Bali, the first province to report a case of HIV in 1987, was placed sixth among Indonesian provinces with the highest cumulative number of HIV cases in 2017. As a popular tourist destination, the spread of genetic variants of HIV through international travel may become a cause for concern in Bali. Tourism is mostly concentrated in South Bali; thus, HIV in less popular regions in North Bali, such as Buleleng Regency, may have different viral characteristics from that in South Bali. Forty-three protease (PR), 40 reverse transcriptase (RT), 27 *gag*, and 23 *env* genes were sequenced from 48 samples derived from ART-experienced individuals. Subtyping revealed CRF01_AE as the dominant circulating recombinant form of HIV-1 in North Bali. Although no major mutation was detected in PR genes, several major mutations were identified in 4 out of the 40 RT genes (10%), indicating the emergence of HIV-1 drug resistance in this region.

Key words: CRF01_AE, ART, North Bali, Indonesia

Text

The Joint United Nations Programme on human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (UNAIDS) estimated that 36.9 million individuals were living with HIV globally in 2017.[1] In December 2017, Indonesia reported 280,623 cumulative cases of HIV infection. The first case of HIV infection in Indonesia was documented in Bali in 1987. With 17,024 cases of HIV infection, Bali was placed sixth among Indonesian provinces with the highest cumulative number of HIV cases.[2]

HIV exhibits large genetic variability due to the high mutation and recombination rates of the reverse transcriptase (RT) enzyme, together with a high rate of virus replication. HIV type 1 (HIV-1), which is responsible for most of the global HIV pandemic, comprises four groups: group M (major), group O (outlier), group N (non-major, non-outlier), and new group P (pending). Group M, the pandemic group of HIV-1, has been further divided into subtypes A to K. Besides these subtypes, circulating recombinant forms (CRFs) and unique recombinant forms (URFs), as a result of recombination between subtypes, have also been identified in group M.[3] To date, 98 CRFs have been recorded in the Los Alamos HIV database (www.hiv.lanl.gov). CRF01_AE, the second predominant circulating CRF accounting for 5% of infection cases worldwide in 2004-2007, is responsible for the vast majority of infections in Southeast Asia, including several regions in Indonesia.[3-7]

Subtypes and CRFs may differ in the rates of disease progression and viral transmission. HIV diversity also affects diagnostic and viral load measurements, and has an impact on responses to antiretroviral treatment (ART) as well as the emergence of drug resistance-associated

mutations;[3] therefore, it is important to monitor circulating HIV-1 subtypes and CRFs in regions for the prevention and control of HIV.

The Indonesian Ministry of Health reported that 91,369 individuals living with HIV/AIDS were being treated ART in December 2017, with 88,386 receiving a first-line regimen and 2,983 receiving a second-line regimen.[2] First-line ART regimens comprise two nucleoside RT inhibitors (NRTIs) and a non-nucleoside RT inhibitor (NNRTI), while two NRTIs plus a ritonavir-boosted protease (PR) inhibitor (PI) are adopted for second-line regimens.[8] ART improves not only the survival rates and quality of life of HIV-infected individuals, but also reduces HIV transmission;[9] however, acquired and transmitted HIV drug resistance may compromise ART.[10] Drug resistance is associated with suboptimal virological suppression, subsequent immunological decline, and poor clinical outcomes.[10]

Travel and tourism are associated not only with HIV-1 dissemination around the world, but also the spread of genetic variants.[11] Bali is a popular tourism destination in Indonesia. Tourism activities in Bali are mostly concentrated in South Bali, including Bandung Regency, Denpasar city, and Gianyar Regency, while Buleleng Regency located in North Bali is a less popular location for international tourism.[12] Previous studies that focused on Gianyar Regency and Denpasar city in Bali identified CRF01_AE as the major cause of infection, and major drug resistance mutations (DRMs) in the RT gene were detected in 25.8% of ART-experienced individuals.[7] The genotypic characteristics of HIV-1 circulating in tourism-concentrated regions may differ from those in regions less popular for tourism; however, limited information is currently available on HIV-1 subtypes and the prevalence of DRM outside South Bali. Therefore, the present study aimed to

characterize HIV-1 subtypes/the distribution of CRFs as well as the appearance of DRM among ART-experienced individuals in Buleleng Regency, Bali.

Ethical approval for the present study was obtained from the Ethics and Law Committee of Universitas Airlangga Hospital (Ethical approval no. 033/KEH/2016) and the Institutional Ethics Committee of Kobe University Graduate School of Medicine (approval no.: 784). Sixty-three HIV-1-infected individuals currently under ART were recruited from the Voluntary Counselling and Testing Clinic of Buleleng Regency General Hospital, Bali. Five milliliters of ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral blood was collected from study participants in July and August 2017, with written informed consent being obtained from each participant prior to the procedure. DNA was then extracted from whole blood samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Demographic and clinical data on study participants were retrieved from medical records and shown in Table 1.

Viral *pol* genes encoding full-length PR and RT genes and partial fragments of the *gag* and *env* genes were amplified from DNA extracted from peripheral blood samples by the nested polymerase chain reaction (PCR) using ExTaq (Takara Bio, Shiga, Japan) and the following primers. The primers DRPRO5, 5′-AGACAGGYTAATTTTTTAGGGA-3′ [corresponding to nucleotides (nt) 2074-2095 of the HIV-1 reference strain, HXB2 (GenBank accession no. K03455)] and DRPRO2L, 5′-TATGGATTTTCAGGCCCAATTTTTGA-3′, (nt 2716 to 2691) were used in first PCR for the amplification of the PR gene, 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. The primers RT1L, 5’-ATGATAGGGGGAATTGGAGGTTT-3’ (nt 2388 to 2410) and GPR2M, 5’-

GGACTACAGTCYACTTGTCCATG-3’ (nt 4402 to 4380) were used in first PCR for the amplification of the RT gene, while RT7L, 5’-GACCTACACCTGTCAACATAATTGG-3’ (nt 2485 to 2509) and GPR3L, 5’-TTAAAATCACTARCCATTGYTCTCC-3’ (nt 4309 to 4285) were used for nested PCR. The primers H1G777, 5’-TCACCTAGAACTTTGAATGCATGGG-3’ (nt 1231 to 1255), and H1P202, 5’-CTAATACTGTATCATCTGCTCCTGT-3’ (nt 2352 to 2328) were used in first PCR for the amplification of the *gag* gene encoding Gag p24, while H1Gag1584, 5’-AAAGATGGATAATCCTGGG-3' (nt 1577 to 1595) and G17, 5’-TCCACATTTCCAACAGCCCTTTTT-3’ (nt 2040 to 2017) were used for nested PCR. The primers M5, 5’-CCAATTCCCATACATTATTGTGCCCCAGCTGG-3’ (nt 6858 to 6889), and M10, 5’-CCAATTGTCCCTCATATCTCCTCCTCCAGG-3’ (nt 7661 to 7632) were used in first PCR for the amplification of the C2-V3 regions of the *env* gene, while M3, 5’-GTCAGCACAGTACAATGIACACATGG-3’ (nt 6948 to 6973), and M8, 5’-TCCTTGGATGGGAGGGGCATACATTGC-3’ (nt 7547 to 7521) were used in nested PCR. Successfully amplified viral genes were then subjected to a sequence analysis performed by Macrogen Japan (http://www.macrogen-japan.co.jp). Sequencing data were assembled and aligned using Genetyx version 10 software (Genetyx, Tokyo, Japan).

The sequencing data of 43 PR genes (297-bp; nt 2253 to 2549), 40 RT genes (1680-bp; nt 2550 to 4229), partial fragments of 27 *gag* genes encoding Gag p24 (381-bp; nt 1627 to 2007), and partial fragments of 23 *env* genes spanning the C2-V3 region (390-bp; nt 7020 to 7409) were obtained from 48 blood samples. We failed to amplify viral genes from the remaining 15 samples presumably due to the low quality of DNA samples. The nucleotide sequences of these PR, RT, *gag*, and *env* genes have been registered in the GenBank database under accession numbers

MK442756-MK442793, MK442795, MK442796, MK442798, MK442800-MK442835, and MK442837-MK442892.

HIV-1 subtyping was conducted using the Recombinant Identification Program (RIP) available on the HIV sequence database website (www.hiv.lanl.gov)[13] and jumping profile Hidden Markov Model (jpHMM) (http://jphmm.gobics.de/submission_hiv).[14] In addition, neighbor-joining (NJ) trees with a Kimura two-parameter model were constructed using MEGA 6.2 software with bootstrap values (1,000 replicates) for relevant nodes being reported on a representative tree. Viral subtyping by RIP and jpHMM was consistent with that by NJ trees (data not shown).

Viral subtyping revealed that 47 samples (97.9%, 47/48) were classified as CRF01_AE, while one remaining sample was classified as a recombinant of CRF01_AE and CRF02_AG (Fig. 1). The results obtained suggested that CRF01_AE is dominant in Buleleng Regency, Bali, similar to other regions in Indonesia as well as in Southeast Asian countries.[3,4,5,6,7] The results of a Blast search (https://blast.ncbi.nlm.nih.gov/) suggested similarities to CRF01_AE circulating in South Bali and Surabaya, as well as other regions of Asia, including Thailand, Vietnam, and China (data not shown).

The appearance of DRMs in successfully sequenced PR and RT genes was investigated according to the International Antiviral Society-USA (IAS-USA) panel.[15] No major mutation was detected in PR genes; however, several drug resistance-associated minor mutations were identified. Among 43 PR genes, 6 (14%) contained L10I/V [amino acid substitution from leucine (L) to isoleucine (I) or valine (V) at position 10 in the PR gene], 15 (34.9%) G16E, 21 (48.8%) K20R/I, 1 (2.3%) L33F, 43 (100%) M36I, 1 (2.3%) I62V, 9 (20.9%) L63P, 40 (93%) H69K, 7 (16.3%) V77I, 8 (18.6%) V82I, 39 (90.7%) L89M/I, and 10 (23.3%) I93L. These mutations potentially

affect viral susceptibility to ritonavir-boosted atazanavir (ATV/r), ritonavir-boosted darunavir (DRV/r), ritonavir-boosted fosamprenavir (FPV/r), ritonavir-boosted indinavir (IDV/r), ritonavir-boosted lopinavir (LVP/r), nelfinavir (NFV), ritonavir-boosted saquinavir (SQV/r), and ritonavir-boosted tipranavir (TPV/r).[15]

Several DRMs were identified in RT genes derived from six patients, four of which showed major mutations. The demographic characteristics of individuals from whom drug resistance-associated major mutations were detected in RT genes are shown in Table 2. The most frequent major mutation found was G190A (7.5%), followed by M184V (5%). Other major mutations with a frequency of 2.5% each were K65R, D67N, K70R, K101E, E138A, Y181C, and Y188L. These mutations were associated with drug resistance to lamivudine (3TC), emtricitabine (FTC), didanosine (ddI), abacavir (ABC), stavudine (d4T), tenofovir (TDF), zidovudine (AZT), rilpivirine (RPV), efavirenz (EFV), etravirine (ETR), and nevirapine (NPV).[15] Minor mutations identified included A98G, V106I, and V179D, with a frequency of 2.5% each. The frequency of drug resistance-associated major mutations in Buleleng Regency in North Bali was lower than that reported previously in Gianyar Regency and Denpasar city of South Bali.[7] Eight out of 31 RT genes (25.8%) isolated in South Bali contained major mutations,[7] in contrast to only four out of 40 RT genes (10%) isolated in North Bali.

In summary, the present study identified CRF01_AE as the dominant circulating CRF in Buleleng Regency, Bali, similar to other regions in Indonesia, including South Bali. The presence of major drug resistance-associated mutations in RT genes suggests the emergence of HIV-1 drug resistance among ART-experienced individuals, particularly those receiving first-line therapy. Any major drug resistance-associated mutation in RT genes needs to be taken into consideration

because the mutation may have an impact on the effectiveness of ART, and continuous monitoring needs to be performed in order to address this issue.

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Author disclosure statement

The authors declare that no competing interests exist.

Sequence Data

Nucleoside sequences are available under GenBank accession numbers MK442756-MK442793, MK442795, MK442796, MK442798, MK442800-MK442835, and MK442837-MK442892.

References

1. Joint United Nations Programme on HIV/AIDS (UNAIDS): UNAIDS Data 2018, 2018. [Accessed on 5 Feb 2019] Available at www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf
2. Direktorat Jenderal Pencegahan dan Pengendalian Penyakit Kementerian Kesehatan RI (Ditjen P2P Kemenkes RI): Laporan Situasi Perkembangan HIV-AIDS & PIMS di Indonesia Januari-Desember 2017, 2018. [Accessed on 5 Feb 2019] Available at http://siha.depkes.go.id/portal/files_upload/Laporan_HIV_AIDS_TW_4_Tahun_2017__1_.pdf
3. Hemelaar J: The origin and diversity of the HIV-1 pandemic. Trends Mol Med 2012;18:182-192.
4. Khairunisa SQ, Kotaki T, Witaningrum AM, et al.: Appearance of Drug Resistance-Associated Mutations in Human Immunodeficiency Virus Type 1 Protease and Reverse Transcriptase Derived from Drug-Treated Indonesian Patients. AIDS Res Hum Retroviruses 2015;31:255-259.
5. Witaningrum AM, Kotaki T, Khairunisa SQ, et al.: Genotypic characterization of human immunodeficiency virus type 1 derived from antiretroviral therapy-naive individuals residing in Sorong, West Papua. AIDS Res Hum Retroviruses 2016;32:812-817.
6. Khairunisa SQ, Ueda S, Witaningrum AM, et al.: Genotypic Characterization of Human Immunodeficiency Virus Type 1 Prevalent in Kepulauan Riau, Indonesia. AIDS Res Hum Retroviruses 2018;34:555-560.

7. Khairunisa SQ, Masyeni S, Witaningrum AM, et al.: Genotypic characterization of human immunodeficiency virus type 1 isolated in Bali, Indonesia in 2016. HIV AIDS Rev 2018;17:81-90.

8. Kementerian Kesehatan Republik Indonesia (Kemenkes RI): Peraturan Menteri Kesehatan Republik Indonesia Nomor 87 Tahun 2014 tentang Pedoman Pengobatan Antiretroviral, 2015. [Accessed on 5 Feb 2019] Available at http://siha.depkes.go.id/portal/files_upload/Buku_Permenkes_ARV_Cetak.pdf

9. Barre-Sinoussi F, Karim SSA, Albert J, et al.: Expert consensus statement on the science of HIV in the context of criminal law. J Int AIDS Soc 2018;21:e25161.

10. Hamers RL, Schuurman R, Sigaloff KC, et al.: Effect of pretreatment HIV-1 drug resistance on immunological, virological, and drug-resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: a multicentre cohort study. Lancet Infect Dis 2012;12:307–317.

11. Perrin L, Kaiser L, Yerly S: Travel and the spread of HIV-1 genetic variants. Lancet Infect Dis 2003;3:22–27.

12. Utama IGBR: Strategi Menuju Pariwisata Bali yang Berkualitas. J Bali Stud 2013;03:69-90.

13. Siepel AC, Halpern AL, Macken C, Korber BT: A computer program designed to screen rapidly for HIV type 1 intersubtype recombinant sequences. AIDS Res Hum Retroviruses 1995;11:1413-1416.

14. Bulla I, Schultz AK, Meinicke P: Improving Hidden Markov Models for classification of human immunodeficiency virus-1 subtypes through linear classifier learning. Stat Appl Genet Mol Biol 2012;11:Article 1.

15. Wensing AM, Calvez V, Gunthard HF, et al.: 2017 Update of drug resistance in HIV-1 infection. Top Antivir Med 2017;24:132-133.

TABLE 1. DEMOGRAPHIC AND CLINICAL DATA OF ART-EXPERIENCED INDIVIDUALS IN BULELENG REGENCY, BALI

		n	Frequency (%)
Sex	M	35	55.6
	F	28	44.4
Age (years)	15-19	1	1.6
	20-24	6	9.5
	25-29	13	20.6
	30-34	11	17.7
	35-39	11	17.7
	40-44	11	17.7
	45-49	6	9.5
	50-54	2	3.2
	55-59	1	1.2
	≥60	1	1.2
ARV regimen	AZT+3TC+NVP	36	57.1
	AZT+3TC+EFV	3	4.8
	TDF+3TC+NVP	3	4.8
	TDF+3TC+EFV	21	33.3
Length of ARV therapy	4-6 months	5	7.9
	6-12 months	11	17.5
	13-24 months	12	19.1
	25-36 months	9	14.3
	37-48 months	10	15.9
	49-60 months	4	6.4
	>60 months	12	19.1
History of tuberculosis co-infection	Yes	18	28.6
	No	45	71.4

AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; EFV, efavirenz; TDF, tenofovir

TABLE 2. DEMOGRAPHIC CHARACTERISTICS OF AND DRUG RESISTANCE-ASSOCIATED MAJOR MUTATIONS IN RT GENES DERIVED FROM ART-EXPERIENCED INDIVIDUALS IN BULELENG REGENCY, BALI

ID	Age (years)	Sex	Subtype[b]	Drug Resistance Mutations[a]		
				NRTI	nNRTI	Resistance
BLL117	33	Male	CRF01_AE	D67N, K70R, M184V	K101E, G190A	Abacavir, Emtricitabine, Lamivudine, Stavudine, Zidovudine, Efavirenz, Nevirapine, Etravirine, Rilpivirine
BLL142	24	Female	CRF01_AE	K65R, M184V	Y181C, G190A	Abacavir, Didanosine, Emtricitabine, Lamivudine, Stavudine, Tenofovir, Efavirenz, Nevirapine, Etravirine, Rilpivirine
BLL156	32	Male	CRF01_AE		G190A	Efavirenz, Nevirapine, Etravirine
BLL183	25	Female	CRF01_AE		E138A, Y188L	Efavirenz, Nevirapine, Etravirine, Rilpivirine

[a]Drug resistance mutations were based on guidelines published by the International Antiviral Society-USA (IAS-USA).

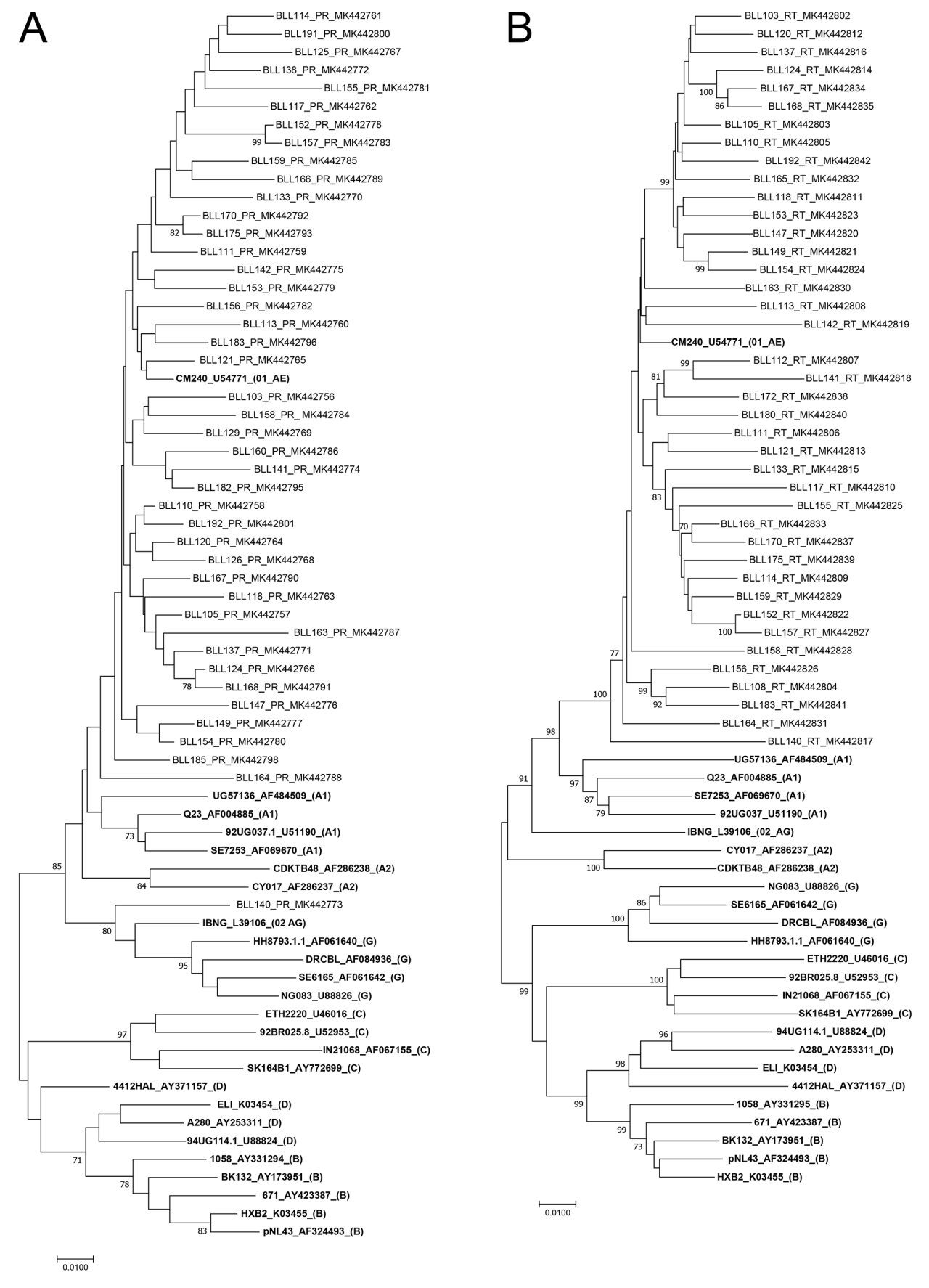
[b]The subtype of the RT gene was assigned based on RIP and phylogenic analyses.

PR, protease; RIP, Recombinant identification program

Figure legends

FIG. 1. Phylogenetic tree analysis of HIV-1 PR, RT, *gag*, and *env* gene sequences collected in Bali, Indonesia.

Phylogenetic trees were constructed for the HIV-1 PR (A), RT (B), *gag* (C), and *env* (D) genes newly sequenced in the present study. The corresponding viral genes of reference HIV-1 strains representing subtypes A1, A2, B, C, D, and G, as well as CRF01_AE (01_AE) and CRF02_AG (02_AG) were included in the analyses (shown in bold). Sequence IDs are presented as a sample ID or the ID of the reference HIV-1 strain, a GenBank accession number, and the subtype or CRF of the reference strain (shown in parentheses) in that order. Bootstrap values were shown if they were >70.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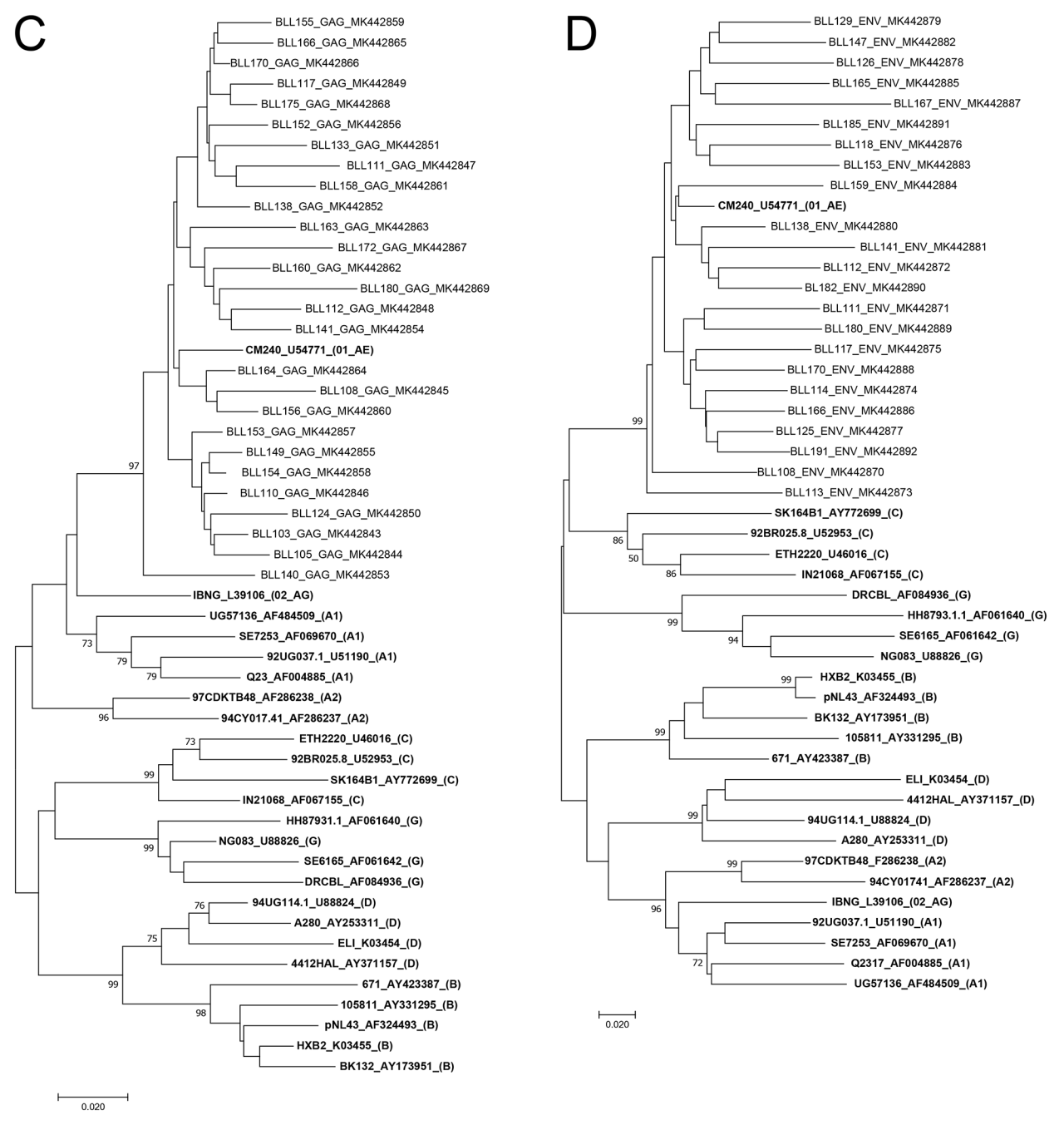
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