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Subtype distribution and drug resistance patterns among HIV-1 strains prevalent in Makassar, Indonesia

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Running head: HIV-1 epidemiology in South Sulawesi, Indonesia.

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Abstract

Human immunodeficiency virus type 1 (HIV-1) is characterized by a large degree of genetic variability because of high rates of recombination and mutation, sizable population sizes, and rapid replication. Therefore, the present study investigated HIV-1 subtype distribution and the appearance of drug resistance mutations (DRMs) in viruses that are prevalent in Makassar, South Sulawesi, Indonesia. The HIV-1 *pol*, *env*, and *gag* genes were amplified from 63 infected individuals and sequenced for a subtyping analysis. CRF01_AE was identified as the predominant HIV-1 circulating recombinant form (CRF) in Makassar, South Sulawesi, Indonesia. Subtype B and recombinant viruses containing CRF01_AE, CRF02_AG, and/or subtype B gene fragments were also detected. Several major DRMs against non-nucleoside reverse transcriptase inhibitors were found among antiretroviral therapy (ART)-experienced subjects, while ART-naive subjects did not possess any transmitted drug resistance. The prevalence of DRMs was very high among ART-experienced subjects; therefore, further surveillance is required in this region.

Key words: HIV-1 subtype, CRF01_AE, HIV drug resistance, Makassar, Indonesia

46 **Text**

47 Human immunodeficiency virus type 1 (HIV-1) infection remains a significant
48 global health issue. HIV-1-infected individuals receive antiretroviral therapy (ART),
49 which is highly effective and recommended for all individuals diagnosed with HIV-1
50 irrespective of their $CD4^+$ T-lymphocyte count. The significant development of
51 antiretrovirals for ART has transformed HIV-1 from an almost uniformly fatal infectious
52 disease into a manageable chronic disease. First-line ART drug regimens consist of a
53 combination of two groups of reverse transcriptase (RT) inhibitors; nucleoside RT
54 inhibitors (NRTI), such as zidovudine and lamivudine (3TC), and non-nucleoside RT
55 inhibitors (NNRTI), including efavirenz (EFV) and nevirapine (NVP), are prescribed in
56 Indonesia.[1]

57 Indonesia is a country with the largest number of HIV-infected cases in Southeast
58 Asia. Based on data reported by the Indonesian Ministry of Health, South Sulawesi was
59 one of the ten provinces with the highest numbers of HIV-infected cases in 2019.
60 Makassar, Pare-Pare, and Kabupaten Janeponto had the highest prevalence of HIV in
61 South Sulawesi province.[2] Makassar is the capital city of South Sulawesi, the fifth largest
62 metropolitan city after Jakarta, Surabaya, Bandung, and Medan in Indonesia, and has the

63 highest HIV infection rate in South Sulawesi. As a result of the HIV epidemic and its
64 integral roles as a metropolitan city, HIV-1 epidemiology needs to be investigated in
65 Makassar. Previous studies identified CRF01_AE as the most prevalent HIV-1 circulating
66 recombinant form (CRF) across the majority of Indonesian cities, including Medan
67 (North Sumatra), Kepulauan Riau, Pontianak (West Kalimantan), Manado (North
68 Sulawesi), Jakarta, Surabaya (East Java), Bali, and Maumere (West Nusa Tenggara).[3] The
69 prevalence of subtype B was reported to be high in West Papua and Papua.[4,5] Nevertheless,
70 data on HIV-1 epidemiology in Makassar remains limited.

71 ART has improved the quality of life of infected individuals and has also
72 decreased mortality and morbidity associated with HIV-1 infection. The emergence of
73 acquired drug resistance (ADR) among ART-experienced subjects and transmitted drug
74 resistance (TDR) among ART-naive subjects are major issues associated with ART. The
75 prevalence of drug resistance mutations (DRMs) amongst ART-naïve HIV-positive
76 pregnant women was estimated to be 2.3–25%, and was recently found to be 24% in
77 freshly-infected juveniles,[6] while the prevalence of TDR in Surabaya was less than 5%.[7]
78 However, data on HIVDR in Makassar remains limited; therefore, surveillance for the
79 emergence of DRMs is needed in the area.

Sixty-three HIV-1-infected individuals were recruited and enrolled in the Voluntary Counseling and Testing program in Makassar Hospital. The present study was approved by the Institutional Ethics Committees of Universitas Airlangga (approval number: 25-995/UN3.14/PPd/2013) and the Kobe University Graduate School of Medicine (approval number: 784). Written informed consent was acquired from all study participants before the execution of this study. Inclusion criteria for recruiting participants to this study were adults older than 18 years, HIV infections confirmed by three diagnostic methods, and ART-experienced for more than one year or ART-naïve. Exclusion criteria were individuals younger than 18 years and pediatric patients.

Whole peripheral blood samples from 63 individuals (60 ART-experienced and 3 ART-naive individuals) were collected into ethylenediaminetetraacetic acid-treated vacutainer tubes. Plasma was separated by centrifugation at 2,000 rpm for 10 minutes. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich, St. Louis, MO, USA). Cellular DNA was isolated from PBMCs using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany). The HIV-1 *pol* gene encoding RT (the RT gene) and protease (the PR gene) as well as the viral *env* and *gag* genes were then amplified using the GoTaq Green

97 Master Mix (Promega, USA) and a pair of specific primers corresponding to the target
98 genes. Primers for amplification and sequencing were the same as those previously
99 described[3] and information is available upon request. Sequencing data collection and
100 alignment were performed using Genetyx software version 10 (Genetyx, Tokyo, Japan).
101 Viral RT, PR, env, and/or gag genes were successfully amplified and sequenced from 48
102 subjects. The nucleotide sequences collected in the present study were registered to the
103 GenBank database with the accession numbers ON244098 - ON244133 (PR genes),
104 ON244134 - ON244168 (RT genes), ON244206 - ON244242 (*env* genes), and ON244169
105 - ON244205 (*gag* genes).

106 HIV-1 subtyping was performed using the recombinant identification program
107 (RIP) available at the HIV sequence database website (http://www.hiv.lanl.gov/) and
108 jumping profile Hidden Markov Model (jpHMM)-HIV (http://
109 jphmm.gobics.de/submission_hiv) on successfully sequenced PR, RT, *gag*, and *env* genes.
110 If the subtype or CRF amongst these genes was inconsistent, the viral gene was
111 considered to be derived from a recombinant virus. In addition, the generation of
112 neighbor-joining (NJ) trees with a Kimura two-parameter model was conducted by
113 MEGA X software. Subtypes A1, A2, B, C, D, and G as well as CRF01_AE and

114 CRF02_AG, as major pandemic subtypes and CRFs of HIV-1, and 3 CRF01_AE/subtype
115 B-recombinants, CRF15_01B, CRF33_01B, and CRF34_01B, as recombinants
116 frequently found in Indonesia, were included in the phylogenetic tree analysis. Sequence
117 information on the representative reference strains of subtypes and CRFs were retrieved
118 from the website
119 (https://www.hiv.lanl.gov/content/sequence/HIV/REVIEWS/RefSeqs2005/RefSeqs05.ht
120 ml). In addition, the identification of DRMs in the PR and RT genes of ART-naïve and
121 ART-experienced subjects was performed manually in accordance with the International
122 Antiviral Society-United States (IAS-USA) guidelines.[8]

123 The demographic characteristics of 63 study participants are shown in Table 1. Of
124 these, 73.0% were male and 27.0% were females. The median age of all patients was 34.2
125 years (range between 21 and 47 years). In addition, 36.5% of patients were of Bugis and
126 Makasar ethnicity. Among these individuals, intravenous drug use and heterosexual
127 intercourse were the two main transmission routes, accounting for 34.9 and 30.2% of
128 infections in the study population. Among 63 individuals, 60 had received ART for longer
129 than one year, while the remaining three were ART-naive. Medication history showed that
130 the majority of ART-experienced individuals had been treated with the combination of

131 3TC, tenofovir, and EFV (49.2%) with an average treatment duration of more than three
132 years.

133 Thirty-eight PR (296 bp), 36 RT (762 bp), 38 *env* (383 bp), and 39 *gag* (369 bp)
134 gene fragments were successfully sequenced from 48 samples and subjected to a
135 phylogenetic analysis. According to the phylogenetic tree, RIP, and jpHMM analyses, the
136 distribution of each subtype and CRF were as follows: 33/48 (68.8%) were CRF01_AE,
137 6/48 (12.5%) were recombinant viruses containing CRF01_AE and CRF02_AG genomic
138 fragments, 4/48 (8.3%) were subtype B, 4/48 (8.3%) were recombinant viruses containing
139 CRF01_AE and subtype B genomic fragments, and 1/48 (2.1%) was a recombinant virus
140 containing CRF02_AG and subtype B genomic fragments (Fig. 1). In the present study
141 CRF01_AE was the predominant CRF in Makassar, Indonesia, similar to other provinces
142 in Indonesia, including Medan, Manado, Surabaya, Jakarta, and Bali.[3]

143 As discovered by the online Genotypic Resistance Interpretation Algorithm
144 (https://hivdb.stanford.edu/hivdb/by-mutations/) and in accordance with IAS-USA
145 guidelines,[8] DRMs were identified in the RT and PR genes. Information on DRMs
146 corresponding to antiretrovirals is listed in Table 2. In RT genes, the percentage of
147 subjects with major and minor DRMs was 5/36 (13.9%). However, ADR in the RT genes

148 was identified in five subjects who were ART-experienced individuals. Only NRTI-
149 associated, not NNRTI-associated DRMs were detected in RT genes. The main DRMs
150 found in RT genes were K103N and E138A, which confer resistance to EFV, NVP, ETR,
151 and RPV. Our previous studies on Pontianak and several provinces in Indonesia revealed
152 the emergence of K103N and E138A among 28.6% (2/7) and 20.0% (8/40) of RT genes
153 derived from ART-treated individuals, respectively.[3,6] In addition, minor DRMs detected
154 in RT genes were V90I and V179D, which confer resistance to ETR. Moreover, major
155 and minor DRMs were detected in two PR genes, MK10 and MK4. The main mutations
156 found were D30N and M46I, which confer resistance to nelfinavir and indinavir/ritonavir.
157 In contrast, no TDR was identified among ART-naive individuals. Minor DRMs,
158 including M36I (33/38, 86.8%), L89M (32/38, 84.2%), K20R/I (27/38, 71.1%), and
159 I93L/M (19/38, 50.0%), were repeatedly detected in PR genes. The aforementioned
160 mutations were discovered to be natural polymorphisms amid CRF01_AE viruses.[8] These
161 results are similar to previous findings showing the presence of minor DRMs affiliated
162 with PR inhibitors in several regions across Indonesia.[3] Nevertheless, treatment outcomes
163 were largely unaffected by the large number of natural polymorphisms found in
164 CRF01_AE.[9]

Based on the present results, CRF01_AE was identified as the dominant HIV-1 CRF in Makasar, Indonesia, similar to other regions in Indonesia. In addition, major and minor DRMs conferring resistance to NRTI were frequently found in RT genes derived from ART-experienced subjects. We consider continuous monitoring for the emergence of HIVDR to be necessary in order to maintain the efficiency of ART and reduce TDR in Indonesia.

Sequence Data

Nucleoside sequences are available under GenBank accession numbers ON244098 - ON244205.

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Author Contributions Statement

S.Q.K., Nasronudin, and M.K. conceived the study. S.Q.K., N.L.A.M., and S.U. performed the experiments. S.Q.K., N.L.A.M., T.K., A.N.H. and M.K. analyzed the data. S.Q.K. drafted the manuscript. All authors reviewed the manuscript.

Author Disclosure Statement

There were no competing financial interests in this study.

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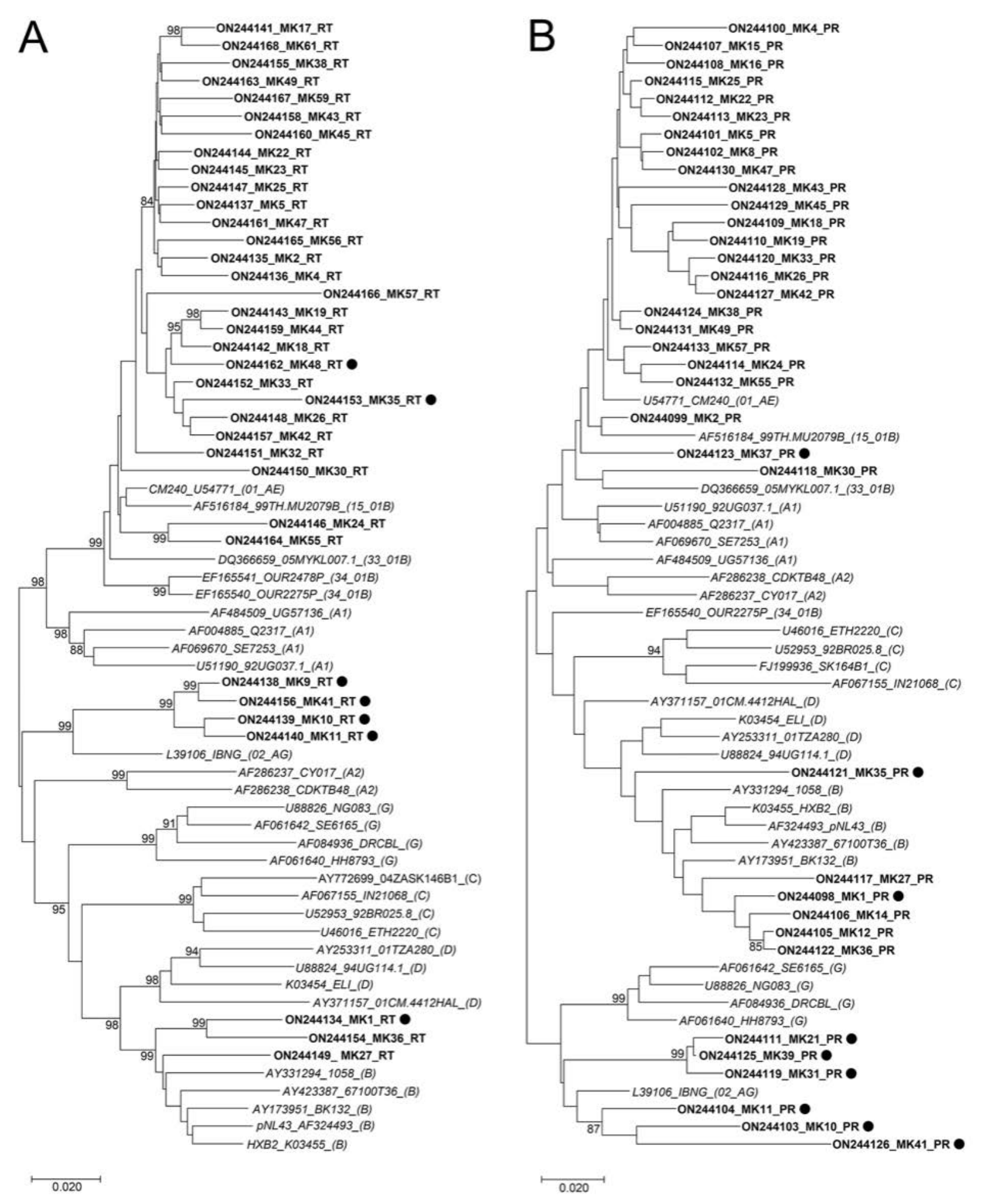
Figure legends

FIG. 1. Phylogenetic tree analysis of HIV-1 RT, PR, *env*, and *gag* genes collected in Makassar, Indonesia.

Phylogenetic trees were constructed for the HIV-1 RT (A), PR (B), *env* (C), and *gag* genes newly sequenced in the present study (D). 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), CRF02_AG (02_AG), CRF15_01B (15_01B), CRF33_01B (33_01B), and CRF34_01B (34_01B) were included in analyses (shown in Italic letters). Sequence IDs are presented as a GenBank accession number, sample ID, or the ID of the reference HIV-1 strain and the subtype or CRF of the reference strain (shown in parentheses) in that order. New sequences identified in the study are highlighted by bold letters, while those derived from recombinant viruses are denoted with circles. Bootstrap values were shown if they were >70.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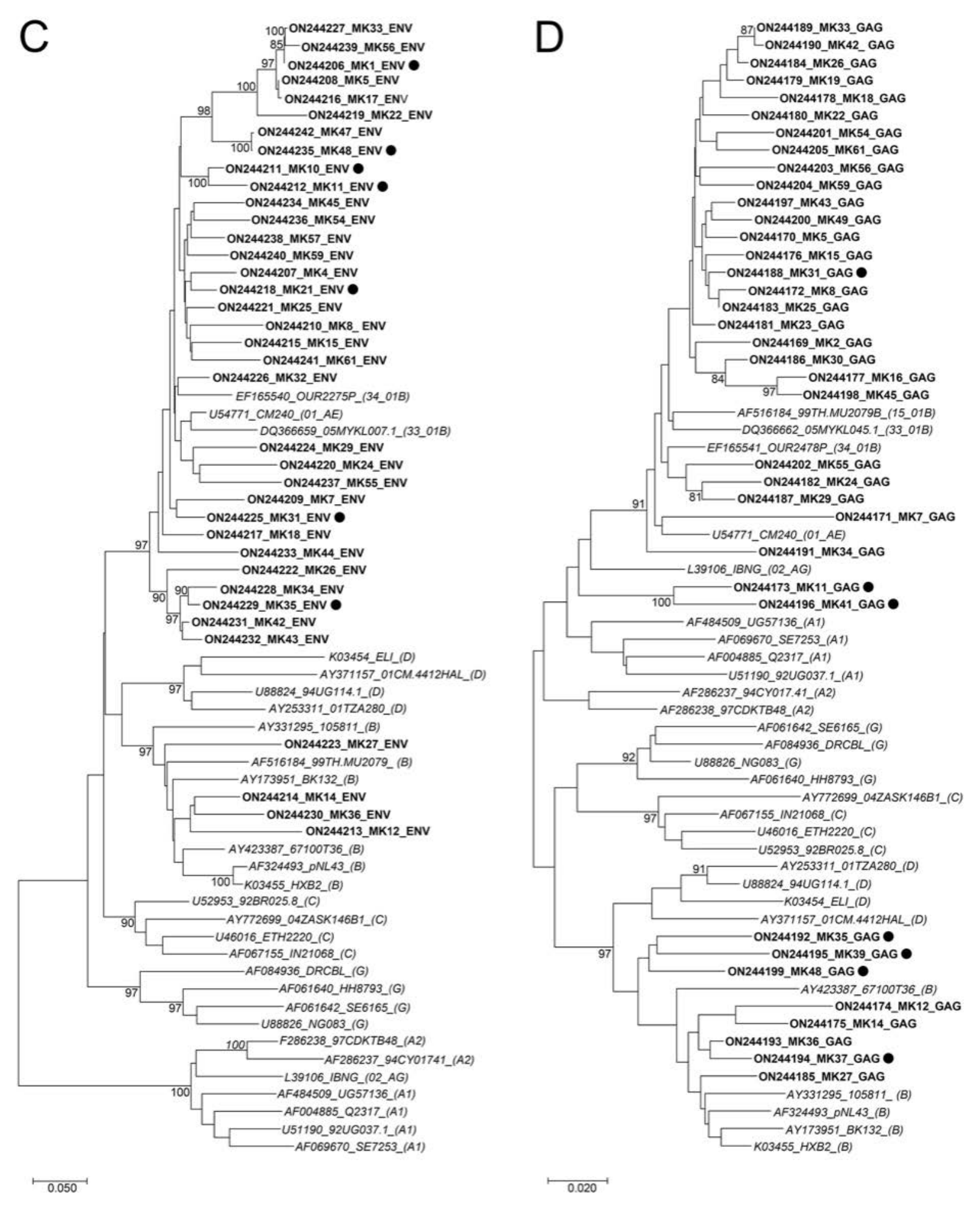
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Table 1. Demographic characteristics of study participants

Characteristics	Value (n = 63)
Age, mean years (SD)	34.2 (6.3)
Sex, n (%)	
Male	46 (73.0%)
Female	17 (27.0%)
Marital status, n (%)	
Married	33 (52.4%)
Single	30 (47.6%)
Ethinicity, n (%)	
Makasar	23 (36.5%)
Bugis	23 (36.5%)
Jawa	5 (7.9%)
Toraja	2 (3.2%)
Batak	1 (1.6%)
Other	9 (14.3%)
Transmission risk category, n (%)	
Heterosexual intercourse	19 (30.2%)
Homosexual intercouse	12 (19.0%)
Intravenous drug use	22 (34.9%)
Commercial sex worker	7 (11.1%)
Unidentified	3 (4.8%)
Types of ART used, n (%)	
3TC+AZT+NVP	13 (20.6%)
3TC+AZT+EFV	15 (23.8%)
3TC+TDF+EFV	31 (49.2%)
3TC+TDF+NVP	1 (1.6%)
Naïve	3 (4.8%)
Oppoturnistic infection, n (%)	
No	49 (77.8%)
Yes	51 (22.2%)

Table 2. Drug resistance mutations and HIV-1 subtypes/CRFs detected in viral genes derived from HIV-1-infected individuals receiving ART

Sample ID	Type of ART; Subtype	Drug Resistance Mutations*			Conferred Resistance to
		NRTI	NNRTI	PI	
MK9	3TC, AZT, EFV; CRF01_AE/CRF02_AG	-	V90I	-	ETR
MK10	3TC, TDF, EFV; CRF01_AE/CRF02_AG-recombinant	-	**K103N**	G16E K20I **D30N** M36I **M46I** L63P L89M I93L	**EFV, NVP**
MK26	3TC, TDF, EFV; CRF01_AE	-	**E138A**	G16E K20R M36I V77I L89M I93L	ETR, **RPV**
MK27	3TC, AZT, NVP; subtype B	-	V179D	L33V I64V I93L	ETR
MK41	3TC, AZT, EFV; CRF01_AE/CRF02_AG-recombinant	-	V90I	G16E K20I **D30N** M36I **M46I** I62V L63P V77I L89M	ETR

* Mutations associated with high resistance according to the guidelines published by the International AIDS Society United States (IAS-USA) are shown. Major mutations are shown in bold.