学位論文

Dynamic HIV-1 genetic recombination and genotypic drug resistance among treatment-experienced adults in northern Ghana
(ガーナ北部のエイズ治療成人患者における HIV-1 組み換えと薬剤耐性)

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Dynamic HIV-1 genetic recombination and genotypic drug resistance among treatment-experienced adults in northern Ghana

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The GenBank /EMBL/DDBJ accession numbers for partial pol sequences of the 24 Ghanaian HIV-1 isolates are LC269861 - LC269884.

Abstract

Purpose: There is hardly any report on HIV-1 drug resistance profile from northern Ghana since antiretroviral therapy (ART) was introduced over a decade ago. This study investigated prevailing HIV-1 subtypes and examined occurrence of drug resistance in ART-experienced patients in Tamale, the capital of the Northern Region of Ghana.

Methodology: A cross-sectional study was carried out on HIV-infected adult patients receiving first-line ART. HIV viral load (VL) and CD4+ T-cells counts were measured. The *pol* gene sequences were analysed for genotypic resistance by an in-house HIV-1 drug-resistance testing; and the prevailing HIV-1 subtypes were analyzed in detail.

Results: A total of 33 subjects were studied. Participants comprised 11 males (33.3%) and 22 (66.7%) females, with median age of 34.5 years (interquartile range [IQR] 30.0–40.3). Median duration on ART was 12 months (IQR 8.0–24). Of 24 subjects successfully genotyped, 10 (41.7%) viruses possessed at least one mutation conferring resistance to nucleoside or non-nucleoside reverse-transcriptase inhibitors (NRTIs/NNRTIs). Two-class drug resistance to NRTI and NNRTI was mostly detected (25%, 6/24). The most frequent mutations were lamivudine-resistance M184V and efavirenz/nevirapine-resistance K103N. HIV-1 subtype CRF02_AG was predominant (79.2%). Other subtypes detected were G (8.3%), A3 (4.2%) importantly two (8.3%) unique recombinant forms with CRF02_AG/A3 mosaic.

Conclusion: HIV-1 shows high genetic diversity and on-going viral genetic recombination in the study region. Nearly 42% of patients studied harboured drug-resistant virus. The study underscores the need for continued surveillance of HIV-1

subtype diversity; and of drug resistance patterns to guide selection of second-line regimens in northern Ghana.

Keywords: Antiretroviral therapy, genotypic resistance, recombinant HIV-1, molecular epidemiology.

Introduction

In Ghana, the HIV prevalence has continued to decline over the past fourteen years, from a peak of 3.6% in 2003, following implementation of strategies by the National AIDS Control Programme (NACP) towards achieving universal access to Anti-retroviral therapy (ART) [1]. The 2016 national HIV prevalence was 1.6%; with regional prevalence ranging from the highest of 2.7% in the Volta and Brong Ahafo Regions to the lowest in the northern regions of the country. Northern region, where this study was conducted, is the largest of the ten regions of Ghana. It has very low population density; it is relatively less resourced and also the region with the lowest HIV prevalence of 0.7% [2–4]. By the end of 2015, since the scale-up began in June 2003, ART services had been expanded to 197 health facilities including 17 private self-financing facilities in 145 out of the 216 districts in all ten regions; and an estimated 89,113 out of 274,562 (~32%) of Persons Living with HIV (PLHIV) and needing treatment, enrolled at the various facilities for ART treatment, care and support [5].

In contrast to its relatively low HIV prevalence, it is striking to note that in the year 2014, for example, the Northern region recorded 4,787 orphaned and vulnerable children, representing 15.5% the total nationwide and the highest recorded in all the ten regions. Besides, enrolment of PLHIV and their clients or dependants for ART service uptake appears low [6]. Until now data is lacking regarding ART outcome in

northern Ghana. Attention and funding for HIV programs in the Northern Region is often less as compared to more population-dense regions and urban centers of Ghana, probably so because of its low ranking in HIV prevalence. Besides, there are certain complex belief systems and practices that constitute barriers in the north, which involve unique traditional beliefs and socio-cultural practices, economics, medicine, psychology and a knowledge gap that affect stigma and discrimination with respect to HIV/AIDS.

At its special Session in 2014 the UNAIDS adopted a post-2015 roadmap - the 90-90-90 targets for ending the AIDS pandemic by 2030. Ghana is a priority country among thirty-five fast track countries identified as accounting for 90% of people newly infected with HIV globally. Besides, the UNAIDS identified Ghana as a priority country for implementation of the 90-90-90 initiative aimed at diagnosing 90% all those infected with HIV globally and reaching 90% of the population of persons living with HIV (PLHIV) with treatment and ensure viralogical suppression in 90% of all patients on treatment by 2020 [7,8]. However, antiretroviral drug resistance emergence and drug-resistant HIV transmission affect the efficacy of ART [9,10] and therefore constitute a formidable hurdle to the attainment of the third -90 target. Thus, to enhance achievement of the targets for ending AIDS, there is need for concomitant ART monitoring.

Surveillance to monitor HIV drug resistance in ART programs remains even more crucial in resource-limited settings where routine HIV drug resistance testing is lacking and assessment of quality-assured virological response to ART is limited [11,12]. Pragmatic measures are therefore necessary especially where resources are limited. Thus, to attain set targets, Ghana adopted a five-year roadmap - The 90-90-90 Ghana Campaign Ending the AIDS Epidemic by 2030 roadmap, that set out national health sector plans to mobilize all stakeholders to locate, test, treat and

retain (L2TR) PLHIV in ART care. The roadmap presents ongoing virologic monitoring as central to attainment of the third -90 target; and underscores HIV VL as the standard parameter for monitoring patients on ART in Ghana. As such, previous drug resistance and VL data could serve as important reference point to decipher trends.

Furthermore, Ghana has a major port/harbour that serves other countries inland of West Africa. There are reports implicating long distance truck drivers as potential transmitters of infections including HIV, due to their long periods of stay away from home [13–15]. Tamale, the region of our study is a major stop over for long distance truck drivers from countries beyond northern Ghana. There is however no report of HIV molecular epidemiology in this region. This study therefore examined virological efficacy and drug resistance profile in adult HIV/AIDS patients in 2010. Concomitantly, the study analyzed in detail the prevailing HIV-1 subtypes to further understand the epidemiology of HIV-1 infections in Ghana.

Methods

Study setting, participants and treatment

HIV-infected patients who accessed antiretroviral treatment service and care at Tamale Teaching Hospital in the Northern Region of Ghana were studied. The study hospital is the regional hospital in Tamale, the capital of the Northern Region of Ghana. It is the third teaching hospital in Ghana after Korle Bu and the Komfo Anokye Teaching Hospitals; and serves as a referral hospital for the three northern regions of Ghana. HIV prevention and intervention programs including provision of free ART services to HIV-infected patients are part of the hospital's health care delivery system. At the time of the study, the estimated number of individuals

accessing ART in the region was about 1047; and about 400 of these were accessing care at the Tamale regional hospital [16]. Of 52 regular attendees who consented to participate, only 33 were found eligible for the study. Some of the factors accounting for low ART uptake in the study area have been discussed in the discussion session.

All ART-receiving patients were assessed using standardized format. ART was initiated in accordance with WHO's recommendations for ART scale up in resource-limited settings. Patients on first-line ART were eligible for the study. A cross-sectional virological efficacy study of patients on ART was carried out between January 2009 and February 2010. The study involved adults (15 years and over) who had received first-line ART for at least 4 months. First-line treatment regimen comprised zidovudine, or stavudine combined with lamivudine, and either nevirapine or efavirenz. Patients who had been off treatment for one month or more were considered to have stopped and so were excluded; and those who were on second-line ART were also excluded in view of the fact that genotypic resistance data were not acquired prior to the switch. Patients were switched to second-line ART mainly by clinical suspicion of virological failure. At enrolment, demographic data, medical history and clinical findings were recorded, which include age, sex, marital status, HIV serostatus, history of antiretroviral use, risk factor for infection, duration on ART (or ART start date), antiretroviral regimen and diagnosed WHO clinical conditions. Laboratory investigations were performed for CD4+ T-cell counts and VL assessment. CD4+ T-cell counts were measured routinely for patients but were not always available, mainly due to resource challenges. Routine VL testing was not available. Ethical approval was obtained from the Institutional Review Board of Noguchi Memorial Institute for Medical Research of the University of Ghana. Patients who consented gave written informed consent to participate in the study.

Immune cell count and plasma HIV-1 viral load assay

To assess immune response, CD4+ T-cells counts were obtained by using a FACSCount flow cytometer (Becton Dickinson, San Jose, California, USA) at the study site. Plasma HIV-1 viral load (pVL) was measured at Noguchi Memorial Institute for Medical Research (NMIMR) using an in-house real-time reverse transcription followed by polymerase chain reaction (RT-PCR) assay with a lower detection limit of 180 copies/mL, according to the method of Barnor et al., 2014 [17]. Patients who had been on ART for more than 3 months and had pVL more than 200 copies/mL were considered as virological failures.

HIV-1 drug-resistance genotyping

HIV-1 drug-resistance genotyping was conducted at the Clinical Research laboratory of the Department of Infection and Immunology, Nagoya Medical Center in Japan. Genotyping was performed as previously reported with some modifications [18]. Briefly, viral RNA was extracted from 200 µL of plasma samples using QIAamp viral RNA mini kit (Qiagen, Hilden, Germany). RT-PCR was performed using QIAGEN one-step RT-PCR kit; and was followed by nested PCR by the use of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, USA) protocol to further amplify the protease (PR) and reverse transcriptase (RT) regions. The primers used were same as previously reported [18]. Generated DNA fragments of 424 bp in PR region (positions 2,168 to 2,591 in the reference HXB2 sequence) and 838 bp of the RT region (positions 2,510 to 3,347) were sequenced using ABI 3730 auto-sequencing system. Sequences were edited with SeqScape software v2.5 (Applied Biosystems)

and HIV-1 drug-resistance mutations were interpreted according to the 2017 resistance mutations update by the International Antiviral Society - USA panel [19].

Phylogenetic and recombination analyses

Twenty-four available isolates comprising 21 PR-RT and 3 RT sequences of the *pol* gene were subtyped. HIV-1 subtyping was initially performed and confirmed separately for the 21 PR-RT fragments (1,095 bp spanning positions 2253 to 3347 of the reference HXB2 sequence) and also for the 3 RT sequence fragments (798 bp spanning positions 2550 to 3347 of the reference HXB2 sequence). However, for the purpose of simplification, the phylogenetic tree presented here was constructed using only RT gene of all 24 isolates in view of similarity in phylogenetic pattern they generate. Phylogenetic tree was constructed by, first, aligning test sequences with references of pure subtypes A-D, F-H, J, K, and all circulating recombinant forms (CRFs) 01 to 88, except 66, 75, 77, 79-84, which were accessed from the HIV Sequence Database of Los Alamos National Laboratory [20] Also included in the subtyping analysis were HIV-1 sub-subtypes A3 (DDI579, DDJ360 and DDJ369) and A4 (97CD_KCC2, 97CD_KTB13 and 02CD_KTB035) isolates, which have been reported to be circulating in some African countries [21,22]. Multiple sequence alignment was then performed by using the MUSCLE program; genetic distances were determined based on the Kimura 2-parameter model; and phylogenetic trees were generated by the neighbour-joining method with 1,000 bootstrap replicates to estimate the reliability of the branching clusters. All phylogenetic and molecular evolutionary analyses were conducted using MEGA version 7 [23].

To clarify recombination, similarity plotting and bootscanning were performed using SimPlot software version 3.5.1 [24] with window and step sizes of 300 and 20 base

pairs respectively. The nine group M HIV-1 phylogenetic subtypes A-D, F-H, J and K; and CRF02_AG were used as references in the SimPlot analyses.

Statistical Analysis

Analysis of statistical significance between categorical and quantitative variables were respectively performed by the Fisher's exact test and the Mann-Whitney U-test programs implemented in GraphPad Prism version 6.07 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). All tests were two-sided with the level of significance set at $P = 0.05$.

Sequence repository

Nucleotide sequences described in this study have been registered and deposited in the DNA databank of Japan under the accession numbers LC269861 - LC269884.

Results

Participants, immunological and virological indices

Thirty-three HIV-1-infected ART-experienced adults (≥15 years old) were studied. The participants comprised 11 males and 22 females; with a median age of 34.5 years (IQR: 30.0-40.3). Table 1 shows details of the demographic and clinical characteristics of the study subjects. The median CD4+ T-cell count was 404 cells/μL. Six patients (18.2%) had CD4+ T-cell count <200 cells/μL. Twenty-three

patients (69.7%) had HIV RNA <200 copies/mL); a total of 28 had <1000 HIV RNA copies/mL; and the remaining 5 patients had VL of >1000 copies/mL. Subjects for whom VL results were obtained were included for drug resistance analyses. CD4+ T-cell count was not available for two of the 33 patients included in the study.

HIV-1 subtype prevalence in Tamale, northern Ghana

Detailed molecular analyses of the HIV-1 *pol* gene sequences were performed to help elucidate the molecular epidemiology of HIV-1 infections in the northern part of Ghana. Nine samples, which had pVL below 200 copies/mL, were unsuccessful in gene amplification despite repeated attempts and by use of alternative, customized primers. There was however no significant difference (Mann-Whitney U-test, p = 0.10628) between the VL of success and failure cases. HIV-1 *pol* gene sequences were therefore available for 24 cases. Phylogenetic analysis, similarity plotting and boot-scanning identified 91.7% (22/24) of the isolates as HIV-1 subtypes and CRFs (Fig. 1). Nineteen out of the 24 isolates (79.2%) were CRF02_AG, 2 (8.3%) were subtype G and 1 (4.2%) was sub-subtype A3. Similarity plotting and boot-scanning analyses (Fig. 2) identified two (8.3%) of the isolates as having unknown mosaic pattern of CRF02_AG/A3 (Fig. 2). For one (Fig. 2(a)), the recombination fragments were 2550-3295 (CRF02_AG) and 3296-3347 (A3), with breakpoint at position 3295; and for the other (Fig. 2(b)), the recombinant fragments were 2550-2668 (CRF02_AG), 2669-2842 (A3), 2843-3216 (CRF02_AG) and 3217-3447 (A3), with breakpoints at positions 2668, 2842 and 3216 (the positions were numbered according to that of the pol gene of HXB2 reference sequence, GenBank accession no. K03455). The two isolates were considered as unique recombinant forms (URFs). In general however, these findings highlight the predominance of HIV-1 CRF02_AG and dynamic viral genetic recombination in Tamale, Ghana.

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258 **HIV-1 drug-resistance mutations among ART-experienced adults in Tamale**

259 All 33 patients studied were treated with the first-line antiretroviral regimen of 2
260 NRTIs + NNRTI; most of who received zidovudine (60.6%) or stavudine (24.2%) with
261 lamivudine and nevirapine (AZT/3TC/NVP or d4T/3TC/NVP). The median duration of
262 ART at the time of the study was 12 months (IQR, 8–24 months). Gene amplification
263 was not successful for 9 samples. Genotypic tests were therefore performed on 24
264 (72.7%, 24/33) of the study subjects. Fourteen (58.3%, 14/24) of the cases tested
265 had no mutations; whereas each of the remaining 41.7% (10/24) had ≥ 1 mutation
266 conferring drug resistance to NRTIs or NNRTIs (Table 2).

267 The most common drug-resistance pattern was 2-class resistance to NRTI and
268 NNRTI (n = 6, 25%), followed by 1-class resistance to either NRTI or NNRTI (n = 2,
269 8% in each case). The most prevalent NRTI and NNRTI mutations were M184V and
270 K103N respectively (n = 6, 25% in each case) (Table 2 A). Two patients' viruses
271 harboured multi-NRTI resistance mutations: one had A62V and the other harboured
272 F116Y and Q151M multi-NRTI mutations in addition to K103N, Y181C and M184V
273 multidrug resistance profile, making this virus resistant to all the NRTIs as well as
274 EFV and NVP (Table 2 B). One patient also had K65R mutation which confers
275 resistance to most NRTIs. Other mutations - E138A in one patient and V90I in four
276 patients, were also detected, which are NNRTI-resistance associated but not
277 responsible for resistance to EFV or NVP prescribed.

278 Considering occurrence of drug-resistant mutations and clinical outcome, one of five
279 patients with virological failure had no drug-resistance mutation (data not shown),
280 which might suggest that virological failure could be due to causes other than
281 acquisition of drug resistance. Cases with virological failure had been on ART for a
282 duration varying from 8 months to 3 years. One case had been on ART for 4 years

with good virological suppression. The cases with or without resistance mutations did not differ significantly in their demographic characteristics.

Discussion

This study presents the prevalence of circulating HIV-1 subtypes and a profile of drug resistance to HIV-1 antiretroviral treatment in Tamale in the Northern Region of Ghana. The findings unambiguously present HIV-1 circulating recombinant form, CRF02_AG as the predominant subtype (79.2%, 19/24) in the region. Previous studies, mainly in the mid-to-southern parts of Ghana, have identified the CRF02_AG as dominating HIV infections in the country [18,25–28]. Data is lacking as to whether this dominance also pertains to the northern parts, which share border with neighbouring Burkina Faso, where CRF06_cpx rather than CRF02_AG is predominant and also other unique forms involving subtypes B, D and K appear to circulate [29,30]. Thus, in agreement with previous reports, the findings of this study adds to data identifying the CRF02_AG as predominating Ghanaian HIV infections, for nearly two decades following its identification in 1997 [18,25–28,31,32]. It is however important to note that some of the studies over the past ten years have identified various URFs [18,27] – an indication of active, on-going viral recombination, which may lead to emergence of a new predominant HIV-1 subtype in the future.

Another important finding of this study is the presence of a unique HIV-1 mosaic form - a recombinant of HIV-1 CRF02_AG and subsubtype A3, which mostly circulate in West Africa. This together with previous data suggests that the CRF02_AG/A3 URF may be a new CRF spreading in Ghana and probably other

307 West African countries. This underscores the importance of continued monitoring of
308 molecular epidemiology and clinical relevance of HIV subtype variations.

309 With respect to outcome of ART in the population under study, the proportion of
310 patients with drug resistance mutations was 41.7%. Four out of five (80%) of patients
311 with virological failure had one or more drug resistance mutations. This observation
312 may suggest drug resistance as the major risk factor for virological failure, which is in
313 conformity with several of previous study findings [33–36]. Adherence data, however,
314 were lacking to enable clarification of this observation. On the other hand, there were
315 a few cases with drug resistance mutations who showed good virological
316 suppression. Considering also the detection of drug resistance mutation in a case as
317 early as 4 months of ART, It is uncertain as to whether drug resistance mutations
318 existed prior to ART initiation, a situation that has been observed in other ART
319 programs [37–40], or mutations developed during treatment. In general, the drug
320 resistance prevalence increased with duration of ART, as 9 resistance mutations
321 were recorded by two years of ART and a total of 25 mutations by 4 years of ART.
322 This feature appears to be common with ART regimen (zidovudine/stavudine
323 lamivudine and nevirapine) commonly used in resource-limited settings [39,41].

324 A number of factors affect access of HIV and AIDS services in the region of the
325 current study; one of which is low level of education and knowledge about HIV/AIDS,
326 which leads to stigmatization and discrimination [42,43]. The 2011 Multiple Indicator
327 Cluster Survey (MICS), Ghana showed that, out of all regions in Ghana, Northern
328 Region ranked second highest in terms of stigmatization and discrimination, or even
329 rejection, of a family member found to have HIV. Comprehensive knowledge about
330 HIV and AIDS is crucial in reducing stigma and discrimination. The Survey revealed
331 that, despite many years of public sensitization, comprehensive knowledge of
332 methods of preventing HIV transmission is higher in more urban areas, which are

usually the more resourced; the highest being in Greater Accra, the capital (47%) and Eastern region (46%), and lowest in Northern region (17%). Thus, intense discrimination, poverty and resulting damaging emotional hurt of HIV-infected individuals have serious consequences that affect HIV transmission and treatment in the Northern Region. Infected persons may develop self-stigma that makes them forego treatment; or reluctant to pick up their drugs, especially from hospital in their locality or region in order to avoid being recognized.

In a broader context, the population of our study did not differ much in terms of the pattern of predominant mutations. The most prevalent resistance mutation pattern observed (which is, dual-class resistance comprising a combination of lamivudine resistance mutation M184I/V with one or more of K103N, Y181C and other mutations conferring resistance to NNRTIs) is similar to that reported commonly in treatment-failure cases in sub-Saharan African countries applying the same first-line treatment [44]. Overall, considering the prevalence of drug resistance mutations observed, nearly half (42%, 10/24) of the treatment cases studied harboured clinically significant resistance mutations; with nearly half (40%, 4/10) of this number being virological failure cases. There is a pressing need for virological monitoring in the resource-limited setting of this study to ensure timely switch to second-line ART and avoid accumulation of drug-resistant viruses, which may worsen the already limited treatment options available.

The results of this study were discussed against a background of some limitations: Data were not collected on adherence, which is an important predictor of resistance to ART [45]; and that limited full appreciation of the relevance of drug resistance mutations observed in relation to clinical outcome of cases. Challenges of sample storage and transportation over long distance to the study laboratory also affected sample recovery for analysis. As such, genotypic data could not be acquired for a

proportion of the samples, mostly those with low VL, which consequently decreased the number of samples suitable for analyses. Furthermore, genotypic data were obtained through direct nucleotide sequencing, which might lack appreciable sensitivity in detecting minority drug-resistant variants obscured by the wild-type strains. Ultra-deep sequencing, which has greater capacity than direct sequencing in detecting not only minority (1%) populations, but also the presence of dual or multiple infections of HIV-1 [46], would be very useful in analyzing HIV in a region where several subtypes of the virus are in circulation. Nevertheless, considering the well-structured nature of ART centres nationwide, and the fact that the findings of this study are in consonance with those reported from previous studies at other sites in Ghana and elsewhere, the above stated limitations could not have significantly impacted the results leading to the study conclusions.

Conclusion

This study documents the occurrence of clinically significant mutations in ART-receiving patients in Tamale in the Northern Region of Ghana. Evidence was found to suggest a failing ART regimen. To the best of knowledge there is hardly any documented study on HIV-1 species characterization and genotypic assessment of ART outcomes in northern Ghana. Accumulation of resistance mutations seriously threatens future treatment options [47]. Therefore the need to strengthen the laboratory infrastructure and personnel, and ensure that laboratories have the capacity for sustainable laboratory monitoring of pVL, CD4+ T-cell counts and HIV drug resistance testing necessary to support successful ART scale-up, cannot be overemphasized. Introduction and use of new sequencing technologies such as next-generation sequencing is needed to better clarify HIV-1 species profile as well as prevalence and transmission of drug resistant HIV-1 variants in Ghana. Finally,

owing to the occurrence of virological failure that accompanies roll-out of ART, there is need for expansion of access to newer antiretroviral drugs from various drug classes to help control HIV/AIDS; not only in Ghana, but in the entire sub-Saharan Africa, which is the most affected region of the world.

Declarations

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Conflicts of Interest

The authors declare that there are no competing interests.

411

412 **Ethical approval and consent to participate**

413 The study protocol was approved by the Institutional Review Board of NMIMR of the
414 University of Ghana. All patients gave written informed consent to take part in the
415 study before blood sample and demographic data were collected.

416

417 **Authors' contributions**

418 Conceived and designed the experiments: NIN SI JAMB WS JSB PB SY TM KY KI
419 WKA; Organized the study team: KI SY TM KY WS WKA. Enrolled patients into the
420 study: PB; Performed the experiments: NIN SI JAMB JSB KI; Prepared a clinical
421 database: NIN SI KI PB; Wrote the paper: NIN SI WKA; Read and approved the final
422 manuscript: NIN SI KI JSB VAB SY TM KY WS WKA.

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Tables, figures and legends

Table 1: Demographic and clinical characteristics of ART-experienced HIV-1-infected patients ≥ 15 years old (n = 33)

Characteristic	n (%)	Median (IQR)
Age (years)		34.5 (30.0 - 40.3)
Gender (n = 33)		
Male	11 (33.3)	
Female	22 (66.7)	
Risk for HIV infection (n = 33)		
Heterosexual	32 (97.0)	
Blood transfusion	1 (3.0)	
†HIV serology (n = 33)		
HIV-1	31 (93.9)	
HIV status not indicated	2 (6.1)	
‡CD4+ T-cell count (cells/µL)		404 (246.8 - 519.0)
>500 cells/µL	9 (27.3)	
200 - 500 cells/µL	16 (48.5)	
<200 cells/µL	6 (18.9)	
§HIV-1 viral load (log_{10} copies/mL)		2.24 (2.23 - 2.38)
3.1 - 4 log	5 (15.2)	
2.3 - 3 log	5 (15.2)	
<200 copies/mL (<2.3 log)	23 (69.7)	
HIV-1 genotype (n = 24)		
CRF02_AG	19 (79.2)	
A3	1 (4.2)	
G	2 (8.3)	
URF	2 (8.3)	
ART regimen (n = 33)		
AZT+3TC+NVP	20 (60.6)	
AZT+3TC+EFV	2 (6.1)	
d4T+3TC+NVP	8 (24.2)	
d4T+3TC+EFV	1 (3.0)	
d4T+EFV	1 (3.0)	
AZT+3TC	1 (3.0)	
Duration of ART (months)		12 (8 - 24)

ART, antiretroviral therapy; AZT, zidovudine; d4T, stavudine; EFV, efavirenz; NVP, nevirapine; 3TC, lamivudine; CRF, circulating recombinant form; URF, unique recombinant form; IQR, interquartile range.

† HIV serology was determined using New LAV Blot I and II (Bio-Rad Laboratories, Marnes-la-Coquette, France).

‡ Data not available for 2 patients

§HIV-1 viral load was measured by the method of Barnor et al [16] system with a detection limit of 180 copies/mL.

Table 2. Characteristics and outcomes of patients with drug-resistant HIV-1

(a) Frequency of HIV-1 drug resistance mutations (N = 24)

Drug class	Mutation	n (%)
Any		10 (41.7)
NRTI resistance		2 (8.3)
NNRTI resistance		2 (8.3)
NRTI and NNRTI resistance		6 (25.0)
None		14 (58.3)
NRTI-resistance mutation		8 (33.3)
	A62V	1 (4.2)
	K65R	1 (4.2)
	F116Y	1 (4.2)
	Q151M	1 (4.2)
	M184V	6 (25.0)
NNRTI-resistance mutation		8 (33.3)
	V90I	3 (12.5)
	K103N	6 (25.0)
	V108I	1 (4.2)
	E138A	1 (4.2)
	Y181C	1 (4.2)
	G190A	1 (4.2)
	H221Y	1 (4.2)
	M230L	1 (4.2)

(b) Patients genotypic and clinical profiles of by duration of ART

ID	ART regimen	Months on ART	Subtype	HIV-1 VL (copies/mL)	Amino acid mutations conferring resistance to* 3TC	EFV or NVP	Any NRTI
o6	AZT, 3TC, NVP	4	CRF02_AG	<180		E138A	
46	AZT, 3TC, NVP	8	CRF02_AG	9123		**K103N**	
NJ-95	AZT, 3TC, NVP	12	CRF02_AG	1164			**A62V**
NJ-117	AZT, 3TC, NVP	17	CRF02_AG	<180	**M184V**	**K103N**	
NJ-139	AZT, 3TC, EFV	22	CRF02_AG	<180			**K65R**
34	AZT, 3TC, EFV	24	CRF02_AG	426	**M184V**	**K103N**, **V108I**	
NJ-133	d4T, 3TC, NVP	24	CRF02_AG	6632	**M184V**	V90I, **K103N**,**Y181C**	**F116Y, Q151M**
NJ-137	d4T, 3TC, NVP	24	URF‡	1071	**M184V**	**K103N**	
63	AZT, 3TC, NVP	36	CRF02_AG	835	**M184V**	V90I, **G190A**, H221Y, **M230L**	
NJ-130§	d4T, EFV	48	CRF02_AG	415	**M184V**	V90I, **K103N**	

VL, viral load; ART, antiretroviral therapy; NRTI, nucleoside reverse-transcriptase inhibitor; NNRTI, non-nucleoside reverse-transcriptase inhibitor; AZT, zidovudine; d4T, stavudine; EFV, efavirenz; NVP, nevirapine; and 3TC, lamivudine.
Amino acid mutations responsible for drug resistance are colour coded and shown in bold.
Amino acid abbreviations: A, alanine; C, cysteine; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; Q, glutamine; R, arginine; V, valine; Y, tyrosine.
*HIV-1 drug-resistance mutations were detected according to the latest definition of the International Antiviral Society-USA panel (15).
‡URF: Unique recombinant form with CRF02_AG/A3 mosaic
§Patient was on two-drug regimen at the time of sampling.

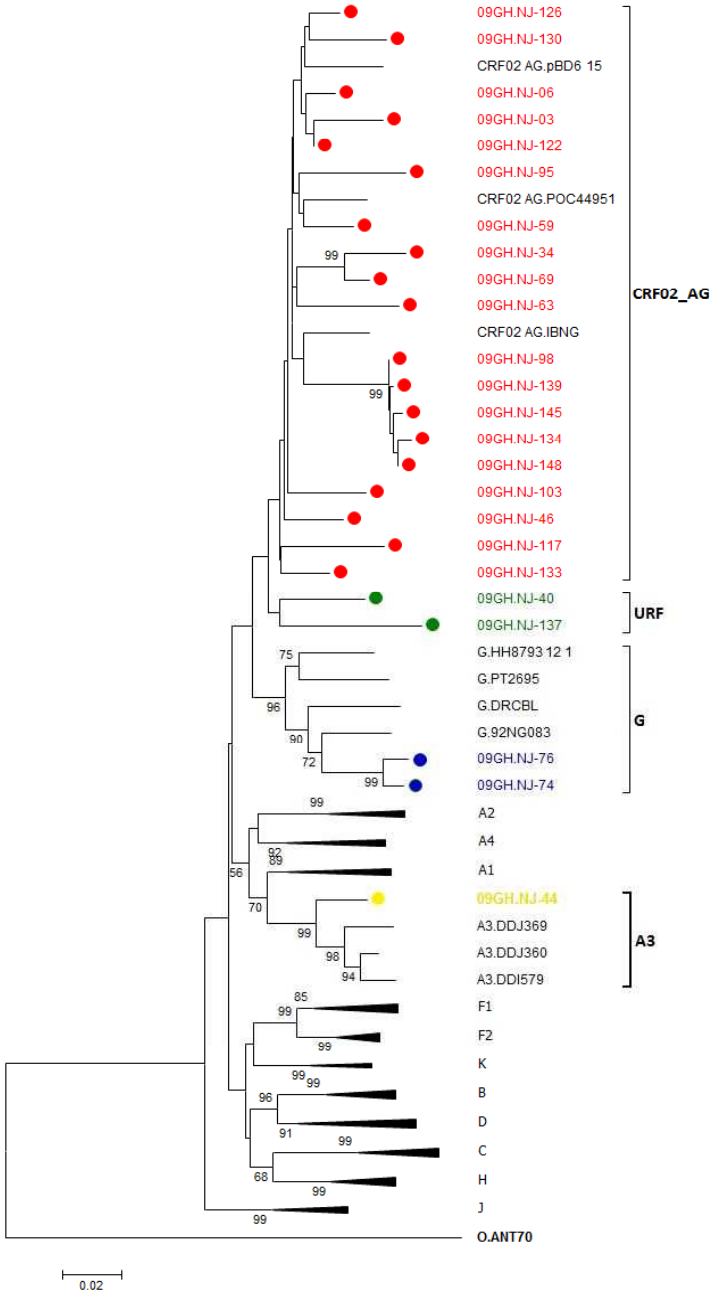

Fig. 1. Molecular epidemiology of HIV-1 infections in Tamale, Ghana. HIV-1 subtypes of 24 Ghanaian isolates were determined through phylogenetic tree construction, similarity plotting and boot-scanning analyses of HIV-1 RT sequences. The evolutionary history was inferred using neighbor-joining method; and the evolutionary distances were computed using Kimura 2-parameter method. Bootstrap values were obtained from 1,000 replicate analyses and values exceeding 70% are shown at tree nodes. The tree displays the Ghanaian isolates classified into known subtypes, CRFs and URFs, which are represented by coloured circles (red for CRF02_AG, blue for subtype G, yellow for subtype A3 and green for URF); and subtype reference isolates, which are represented by the subtype and isolate name. The scale represents the number of nucleotide substitutions per site. HIV-1 group O isolate, ANT70, was used as the outgroup. Evolutionary analyses were conducted in MEGA7. RT, reverse transcriptase; CRF, circulating recombinant form; and URF, unique recombinant form.

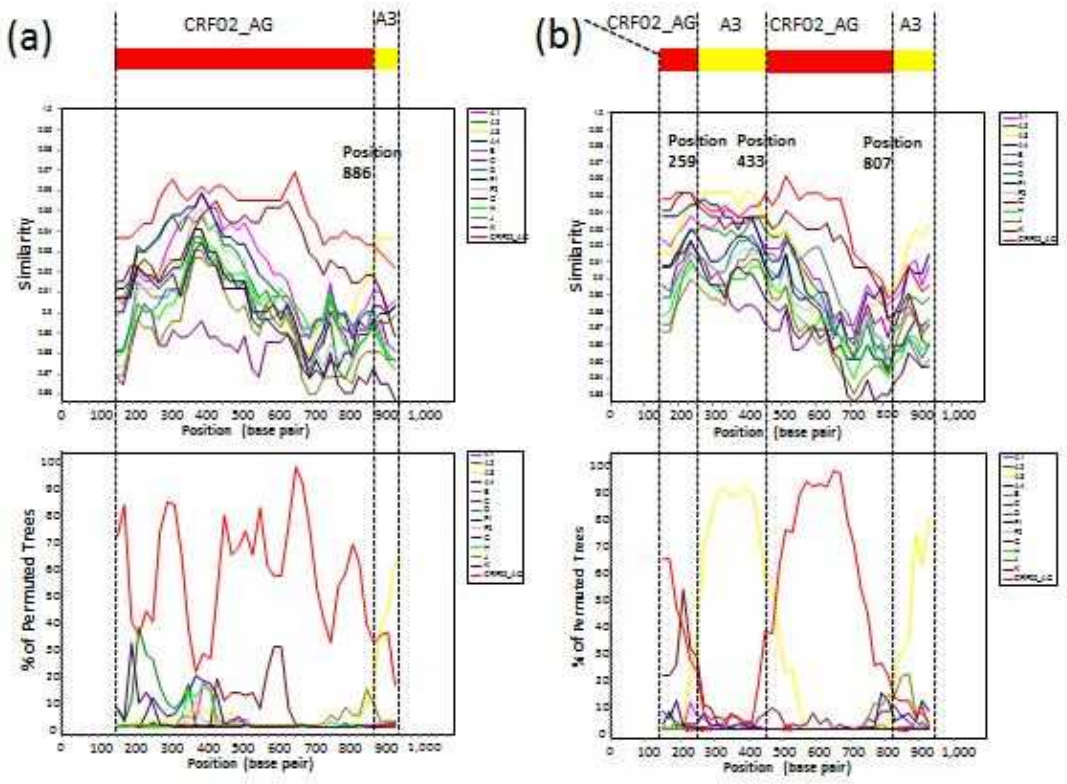

Fig. 2. SimPlot analyses of the *pol* gene sequences of two Ghanaian HIV-1 isolates 09GH.NJ-40 (Left, 2a) and 09GH.NJ-137 (Right, 2b). Upper panel shows similarity plots comparing sequence relationships of the two viruses to representatives of reference subtypes A-D, F-H, J, K and CRF02_AG obtained from the Los Alamos database (https://www.hiv.lanl.gov). The Y-axis represents the percentage of sequence similarity to the corresponding subtype. Lower panel is the result of bootscan analyses, showing plots of bootstrap values (percentage permuted trees) calculated from multiple genome alignment of test viral sequences with reference subtype sequences. Bootscanning was performed using the neighbour-joining algorithm modelled with Kimura-2 parameter method for 100 replicates. Similarity plotting and bootscanning were generated in SimPlot version 3.5.1 with parameter settings of simple consensus sequences; and window and step sizes of 300 and 20 nucleotides respectively. For all panels the *x* axis indicates the nucleotide positions along the alignment (gaps were removed from the alignment). Reference sequences are colour-coded and listed on the right of each plot. Points of crossover of the two curves indicate recombination breakpoints. The analyses confirm the presence of a unique recombinant profile of CRF02_AG/A3.