HIV-2, but as in HIV-1 infection HIV-2 can be isolated from virtually all HIV-2-seropositive patients with AIDS [8–10]. Most of these viruses show early and high reverse transcriptase (RT) activity and exhibit a syncytium-inducing (SI) phenotype in peripheral blood mononuclear cell (PBMC) cultures [8]. It has been reported that cytopathic HIV-2 strains are only found within subtype A and that attempts to isolate viruses belonging to HIV-2 subtypes C, D and E and to a lesser extent subtype B, usually remain unsuccessful [11].

Here we describe data on the pathogenesis and characteristics of viruses isolated from 11 HIV-2-infected individuals living in the Rotterdam area, of whom nine belonged to West African immigrant communities. In addition, the same data are presented for one HIV-2-infected individual born and living in France. By comparing the number of seropositive individuals with and without progressive disease among HIV-1 and HIV-2-infected individuals of West African origin in the Rotterdam area, it can be noted that there is a similar pathogenicity between the predominantly involved HIV-2 subtype A and HIV-1 in the same community.

Materials and methods

Study population

From August 1989, clinical and immunological parameters, and from August 1994, virological parameters were monitored in 11 HIV-2-seropositive individuals and one individual with dual HIV-1 and HIV-2 infection who regularly visited clinics in Rotterdam or Paris. All individuals gave informed consent and the study was approved by the medical ethical committee of the Rotterdam hospital.

Age, sex, country of birth and probable mode of transmission in these individuals were recorded. Nine out of 12 individuals originated from West Africa. Individual RH-2-4 was a Dutch-born male spouse of individual RH-2-5, who was born in the Cape Verdian Isles. RH-2-11 was a Dutch-born former regular female sexual partner of a Cape Verdian Isles man (serostatus unknown). Patient PH-2-1 was a French-born homosexual man living in Paris with documented HIV-2 seroconversion in 1992.

Two patients had been treated with zidovudine for 2 years (RH-2-2) and 6 months (RH-2-5) at the moment of virus isolation.

All patient sera tested negative for HIV-1, human T-cell leukaemia virus-I, hepatitis B surface antigen, except for individual RH-2-8, who was infected with both HIV-1 and HIV-2.

Serology and virus isolation

Sera were tested for antibodies to HIV-1 and HIV-2 (ELFA, BioMerieux, Lyon, France), and by type-specific Western blot (HIV-Blot 2.2, Diagnostic Biotechnology, Leuven, Belgium and New LAV Blot 2, Sanofi Diagnostics Pasteur, Maassluis, The Netherlands).

PBMC were isolated by Ficoll density centrifugation as previously described [12]. All PBMC samples were cocultured for 3 days with phytohaemagglutinin (PHA)-prestimulated donor seronegative lymphocytes (PHA-PBMC) according to standard protocols [12]. All cultures were maintained for up to 6 weeks and monitored for the presence of HIV p24 antigen (V5 ELISA, Organon Teknika, Oss, The Netherlands) in the supernatant at weekly intervals. T-cell lines (MT2, SupT1, H9) and monocyte-derived macrophages were infected with culture supernatants of the HIV-2-positive PBMC cultures, which were twice-weekly analysed for the presence of p24 antigen in supernatants and syncytium formation [13]. HIV-1_{IIIB}, kindly provided by the Medical Research Council AIDS-directed programme [14], and HIV-1 strain ACH 172.BA-L (NSI), provided by Dr H. Schuitemaker from the Central Laboratory for Blood Transfusion Service in Amsterdam [15], were used for reference purposes.

Polymerase chain reaction detection of RT sequences and phylogenetic analysis

High molecular-weight DNA was extracted from MT2 cells that had been cocultured with PBMC from patients RH-2-2, RH-2-2, RH-2-6 and RH-2-7, or directly from PBMC from patients RH-2-1 and PH-2-1 using Celite beads (Janssen Chimika, Beersen, Belgium) [16]. Amplification was performed as previously described [11]. Briefly, RT sequences were amplified in a nested polymerase chain reaction with primers RTC/RT2 and RT3/RT4 for 1.5 min at 94°C, 1 min at 40°C, 2 min at 74°C for 30 cycles with a final extension of 10 min. The amplified fragments were cloned in pTA cloning vector (Invitrogen, San Diego, California, USA) according to the manufacturer's protocol. Clones were sequenced with the *Taq* Dye Deoxy Terminator sequencing kit on the 373A sequencing system of Applied Biosystems (Foster City, California, USA). All clones were sequenced on both strands using custom-made primers, except for a small (200-base-pair) part of RH-2-2, to which none of the reverse primers hybridized.

The phylogenetic relationships of the viruses were determined from their RT nucleotide sequences using the unweighted pair group method with arithmetic mean (UPGMA) (Geneworks; Intelligenetics, Mountain View, California, USA). Alignment of the protein sequences was performed with the Geneworks software.

Results

Clinical status and serology

The clinical status of the 12 HIV-2-seropositive individuals at entry in the study is shown in Table 1. According to the revised classification system of the Centers for Disease Control and Prevention [17] five patients (RH-2-2, RH-2-6, RH-2-9, RH-2-10, RH-2-11) were classified in category C3, one (RH-2-1) in category C1, two (RH-2-5, RH-2-7) in category A3 and three (RH-2-3, RH-2-4, RH-2-8) in category A1. RH-2-2 had a history of tuberculosis, recurrent oral candidiasis and cytomegalovirus (CMV)-induced retinitis. RH-2-6 suffered from extreme wasting. RH-2-9 had intracerebral malignant lymphoma, RH-2-10 was diagnosed with disseminated *Mycobacterium avium-intracellulare*, and RH-2-11 had a history of *Pneumocystis carinii* and CMV retinitis. All five of them suffered from end-stage AIDS at the time of entry in the study. RH-2-1 had suffered from Kaposi's sarcoma since 1989, which was localized on the lower extremities. The other six patients were asymptomatic at entry.

For eight of the individuals no information was available about the time of seroconversion. From three patients (RH-2-9, RH-2-10, RH-2-11) PBMC were not available. One individual (PH-2-1) was studied for 36 months from the time of seroconversion, during which period his CD4+ cell counts decreased from about 600 to 160×10^6/l (Fig. 1). No overt clinical signs developed in this patient during the observation period. Western blot analysis of his serum samples collected during the first 16 weeks after seroconversion showed that antibodies against all major HIV-2 proteins developed (Fig. 1).

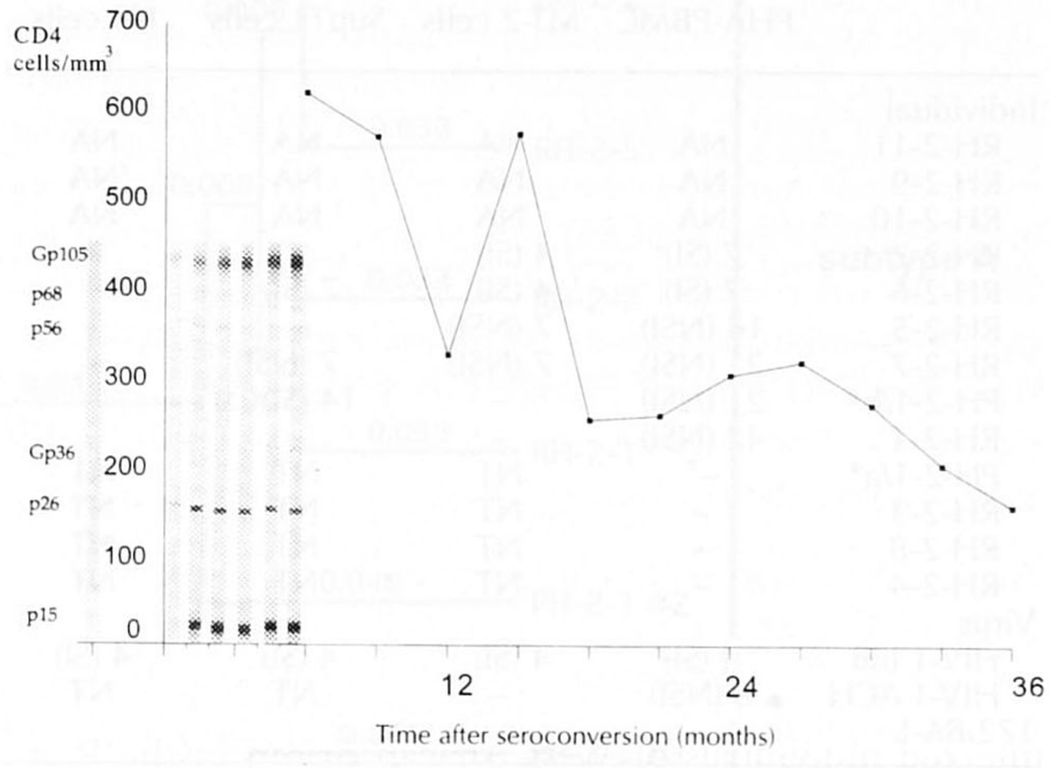

Fig. 1. Sequential Western blot profiles of HIV-2-specific serum antibodies in patient PH-2-1 during seroconversion in October 1992, and his CD4+ cell counts during the 36-month period after seroconversion.

Comparison of clinical and immunological status of HIV-1-infected individuals of West African origin in the Rotterdam hospital during the same study period showed that nine out of 17 HIV-1-infected individuals suffered from progressed HIV-related disease at the time of first presentation in the clinic (Table 1). The mean age

Table 1. Demographic and clinical characteristics of the study participants at entry.

Subject	Age (years)	Sex	Country of birth	Probable mode of transmission	Disease state (CDC criteria)	CD4 count ($\times10^6$/l)	Serology	
							HIV-1	HIV-2
RH-2-11	54	Female	Netherlands	Heterosexual	C3	10	–	+
RH-2-9	35	Female	CVI	Heterosexual	C3	10	–	+
RH-2-10	35	Male	CVI	Heterosexual	C3	10	–	+
RH-2-2	36	Male	CVI	Heterosexual	C3	10	–	+
RH-2-6	34	Female	Ghana	Heterosexual	C3	10	–	+
RH-2-5	40	Female	CVI	Heterosexual	A3	110	–	+
RH-2-7	39	Male	CVI	Heterosexual	A3	130	–	+
PH-2-1	50	Male	France	Homosexual	A1	570	–	+
RH-2-1	38	Male	CVI	Heterosexual	C1	600	–	+
RH-2-3	36	Female	CVI	Heterosexual	A1	800	+	+
RH-2-8	35	Female	Ghana	Heterosexual	A1	1000	–	+
RH-2-4*	54	Male	Netherlands	Heterosexual	A1	1200	–	+
RH-1-1	51	Male	CVI	Heterosexual	C3	10	+	–
RH-1-2	36	Female	CVI	Heterosexual	C3	10	+	–
RH-1-3	28	Male	Ivory Coast	Heterosexual	C3	10	+	–
RH-1-4	35	Female	Ghana	Heterosexual	C3	10	+	–
RH-1-5	31	Male	Senegal	Heterosexual	C3	50	+	–
RH-1-6	36	Male	CVI	Heterosexual	C3	60	+	–
RH-1-7	27	Male	CVI	Heterosexual	C3	80	+	–
RH-1-8	27	Male	CVI	Heterosexual	C3	90	+	–
RH-1-9	35	Male	Ghana	Heterosexual	A3	170	+	–
RH-1-10	29	Male	CVI	Heterosexual	A2	220	+	–
RH-1-12	37	Female	CVI	Heterosexual	A2	350	+	–
RH-1-13	29	Female	CVI	Heterosexual	A2	400	+	–
RH-1-14	45	Male	CVI	Heterosexual	A1	510	+	–
RH-1-15	36	Female	CVI	Heterosexual	A1	580	+	–
RH-1-16	37	Female	CVI	Heterosexual	A1	750	+	–
RH-1-17	31	Male	Ghana	Heterosexual	A1	860	+	–

*Husband of RH-2-5. CVI, Cape Verdian Islands.

Table 2. HIV-2 isolation and *in vitro* characteristics.

	Day of first p24 antigen detection upon infection of			
	PHA-PBMC	MT-2 cells	SupT1 cells	H9 cells
Individual				
RH-2-11	NA	NA	NA	NA
RH-2-9	NA	NA	NA	NA
RH-2-10	NA	NA	NA	NA
RH-2-2	7 (SI)	4 (SI)	–	–
RH-2-6	7 (SI)	4 (SI)	7 (SI)	–
RH-2-5	14 (NSI)	7 (NSI)	–	–
RH-2-7	21 (NSI)	7 (NSI)	7 (NSI)	–
PH-2-1/b*	21 (NSI)	–	14 (SI)	–
RH-2-1	42 (NSI)	–	–	–
PH-2-1/a*	–†	NT	NT	NT
RH-2-3	–	NT	NT	NT
RH-2-8	–	NT	NT	NT
RH-2-4	–	NT	NT	NT
Virus				
HIV-1 IIIB	4 (SI)	4 (SI)	4 (SI)	4 (SI)
HIV-1 ACH 172.BA-L	6 (NSI)	–	NT	NT

*PH-2-1/a, 7 months after seroconversion, CD4+ cell count 570×10^6/l; PH-2-1/b, 36 months after seroconversion, CD4+ cell count 200×10^6/l. †No p24 antigen demonstrated within 6 weeks of incubation. PHA-PBMC, Phytohaemagglutinin-stimulated peripheral blood mononuclear cells; SI, syncytium-inducing; NSI, non-syncytium-inducing; NT, not tested; NA, no materials available.

of the HIV-1 infected population was 32 years, and the probable mode of transmission was heterosexual contact.

Virus isolation

HIV-2 was isolated in PHA-PBMC from the PBMC of six of the eight individuals with CD4+ cell counts ranging from 600 to 10×10^6/l (Table 2). The time of first HIV p24 antigen detection in culture supernatants, which ranged from 7 to 42 days after inoculation, correlated inversely with the patients CD4+ cell counts at the time of sampling ($r = 0.95$, $P = 0.01$). The rapidly replicating HIV-2 isolates from patients RH-2-2 and RH-2-6, who suffered from end-stage AIDS, induced syncytia in the PHA-PBMC, whereas the other four HIV-2 isolates failed to do so. The four HIV-2 isolates from the patients with the lowest CD4+ cell counts ($130–10\times10^6$/l) were shown to replicate in MT2 cells. The two isolates from the end-stage AIDS patients RH-2-2 and RH-2-6 induced syncytia and p24 antigen production after 7 days (Table 2). Isolates from RH-2-6, RH-2-7 and PH-2-1, who all had CD4+ counts < 200×10^6/l, replicated in SupT1 cells as shown by the presence of detectable p24 antigen in the supernatants within 14 days. The HIV-2 isolates from patients

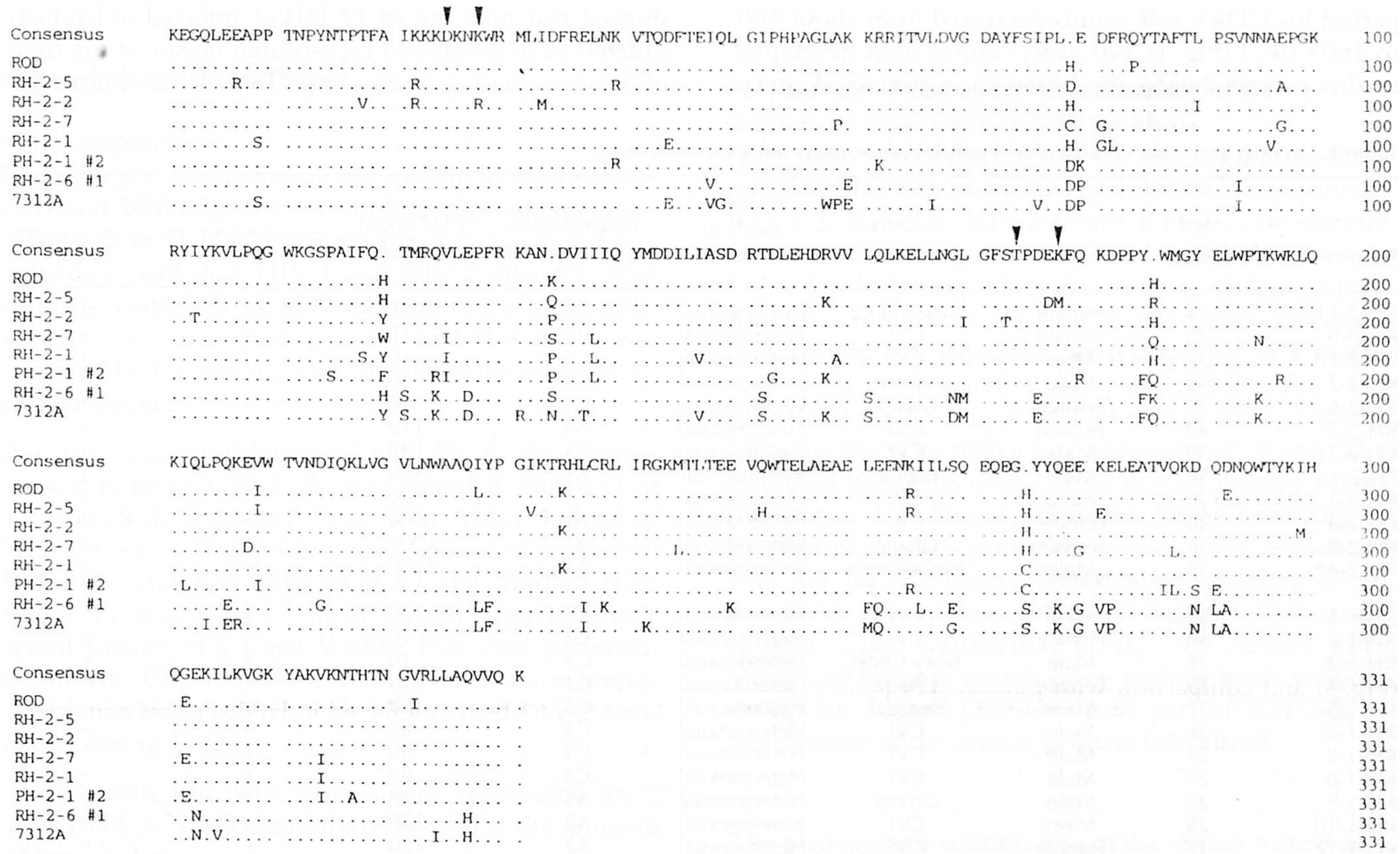

Fig. 2. Alignment of reverse transcriptase (RT) protein fragment sequences from six HIV-2 viral isolates. The nucleotide sequences were determined from polymerase chain reaction (PCR) amplification products, which were translated and aligned; ROD (HIV-2 subtype A) and 7312A (HIV-2 subtype B) were used for reference purposes. The arrows (▼) indicate mutations corresponding with zidovudine resistance in HIV-1.

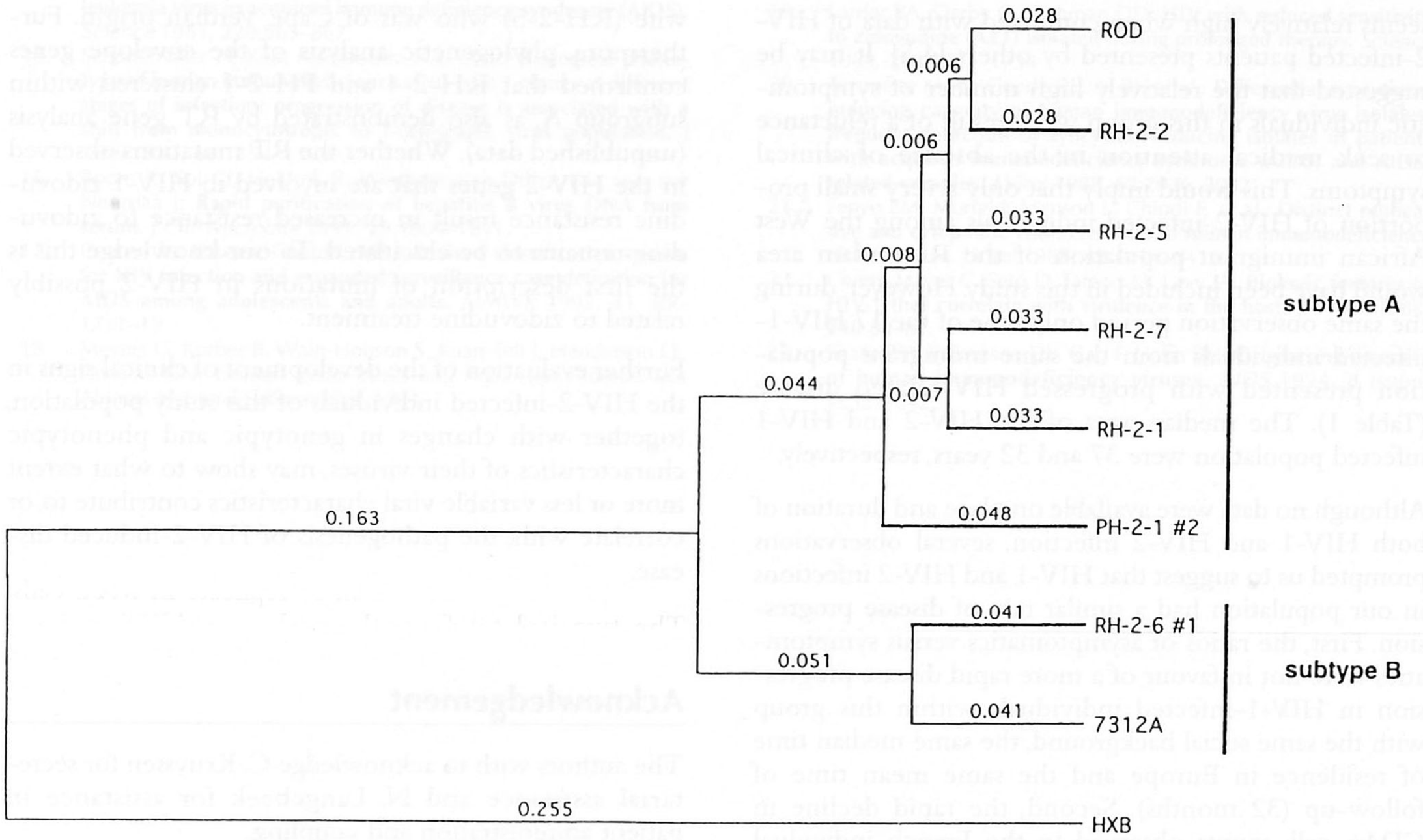

Fig. 3. Phylogenetic tree of HIV-2 isolates estimated from their nucleotide sequences of reverse transcriptase (RT) fragments (unweighted pair group method with arithmetic mean; UPGMA). Strains ROD (HIV-2 subtype A), 7312A (HIV-2 subtype B) and HXB (HIV-1) were used for reference purposes.

RH-2-6 and PH-2-1 also induced syncytia in these cells (Table 2). No replication could be demonstrated with any of the HIV-2 isolates in H9 cells.

Polymerase chain reaction amplification of RT gene sequences

To further characterize the HIV-2 isolates, RT gene segments were amplified and sequenced. The open reading frames from all the clones that were fully sequenced (n = 11) were intact with the active YMDD site present and no stop codons observed. Fig. 2 shows the alignment of the deduced amino-acid sequences of one clone for each of the six HIV-2 isolates. Limited amino-acid diversity was observed in the individuals originating from the Cape Verdian Isles (91–94% identity). Phylogenetic analysis indicated that all these isolates grouped together (Fig. 3) and comparison with published sequences indicated that they should be included into subtype A [11,18]. The French patient who had probably been infected by an African immigrant in France of unknown origin also grouped within subtype A (89–92% identity with the subtypes A from the Rotterdam area). The HIV-2 isolate from patient RH-2-6, who was of Ghanese origin, proved to be more distantly related (84–86% identity with the subtype A isolates) and grouped within subtype B [11], which was confirmed by bootstrap analysis.

Mutations related to zidovudine resistance

RT fragments corresponding to the region that is implicated in zidovudine resistance in HIV-1 [19] were amplified for the two HIV-2 isolates from patients who had been treated with zidovudine for 2 years (RH-2-2) and 6 months (RH-2-5). In HIV-2 isolate RH-2-2 mutation $K_{28} \rightarrow R_{28}$ and in isolate RH-2-5 mutation $K_{177} \rightarrow M_{177}$ were identified. The former change has also implicated in HIV-1 zidovudine resistance, and the latter may be specific for HIV-2 isolates.

Discussion

In this study we have presented clinical and virological data from 12 HIV-2-infected individuals living in western Europe. Nine of them were of West African origin and three were born in western Europe. All except one (PH-2-1) had probably been infected through heterosexual contact. Seven of the West African patients presented with an AIDS indicator disease or a CD4 cell count $< 200 \times 10^6$/l. Therefore, the number of HIV-2-infected patients of West African origin with progressed HIV-related symptoms upon presentation to the Rotterdam hospital was seven out of nine (Table 1). This ratio

seems relatively high when compared with data of HIV-2-infected patients presented by others [4,5]. It may be suggested that the relatively high number of symptomatic individuals in this group was a result of a reluctance to seek medical attention in the absence of clinical symptoms. This would imply that only a very small proportion of HIV-2-infected individuals among the West African immigrant population of the Rotterdam area would have been included in this study. However, during the same observation period only nine of the 17 HIV-1-infected individuals from the same immigrant population presented with progressed HIV-related disease (Table 1). The median ages of the HIV-2 and HIV-1 infected population were 37 and 32 years, respectively.

Although no data were available on place and duration of both HIV-1 and HIV-2 infection, several observations prompted us to suggest that HIV-1 and HIV-2 infections in our population had a similar rate of disease progression. First, the ratios of asymptomatics versus symptomatics were not in favour of a more rapid disease progression in HIV-1-infected individuals within this group with the same social background, the same median time of residence in Europe and the same mean time of follow-up (32 months). Second, the rapid decline in CD4+ cell counts observed in the French individual (PH-2-1) over a 36-month observation period clearly showed that HIV-2 infection may also run a rapidly progressive course. This is not in agreement with the relatively slow disease progression reported by others for HIV-2-infected individuals [4,5]. So far we could not identify any predisposing factors, such as coinfection with other blood-borne viruses or a selective immigration pattern among these individuals (data not shown).

The results of virus isolation studies in PHA-PBMC and T-cell lines, indicating more successful virus isolation from individuals with progressed disease and low CD4+ cell counts, are in agreement with the data presented by others [8,20–22]. The correlation between an SI virus phenotype in PHA-PBMC and in MT2 cells, with more progressed disease or lower CD4+ cell counts, or both, is similar to observations in HIV-1-infected individuals [20–22]. This correlation indicates that, as in HIV-1 infection, this marker may be of prognostic value.

No exact information about geographical origin of the HIV-2 infection of the individuals in our study could be obtained. Nevertheless, in the phylogenetic analysis of the RT gene segments all the viruses from individuals of Cape Verdian origin clustered within subtype A. HIV-2_{ROD}, which also originated from an individual of Cape Verdian origin [23], also clusters within this subtype. This phylogenetic analysis also indicated that the HIV-2 isolate from the individual born in Ghana (RH-2-6), clustered within subtype B. This is the first subtype B strain reported to have a SI phenotype. Preliminary sequencing data of the envelope genes of RH-2-4, RH-2-5 and PH-2-1 indicate that individual RH-2-4, who was born in The Netherlands, was infected by his wife (RH-2-5) who was of Cape Verdian origin. Furthermore, phylogenetic analysis of the envelope genes confirmed that RH-2-4 and PH-2-1 clustered within subgroup A, as also demonstrated by RT gene analysis (unpublished data). Whether the RT mutations observed in the HIV-2 genes that are involved in HIV-1 zidovudine resistance result in increased resistance to zidovudine remains to be elucidated. To our knowledge this is the first description of mutations in HIV-2 possibly related to zidovudine treatment.

Further evaluation of the development of clinical signs in the HIV-2-infected individuals of this study population, together with changes in genotypic and phenotypic characteristics of their viruses, may show to what extent more or less variable viral characteristics contribute to, or correlate with, the pathogenesis of HIV-2-induced disease.

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