



A phylogenetic follow-up study of 4 individuals
infected with closely related HIV-1 strains

MIEP

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Introduction

HIV is the causal agent of AIDS

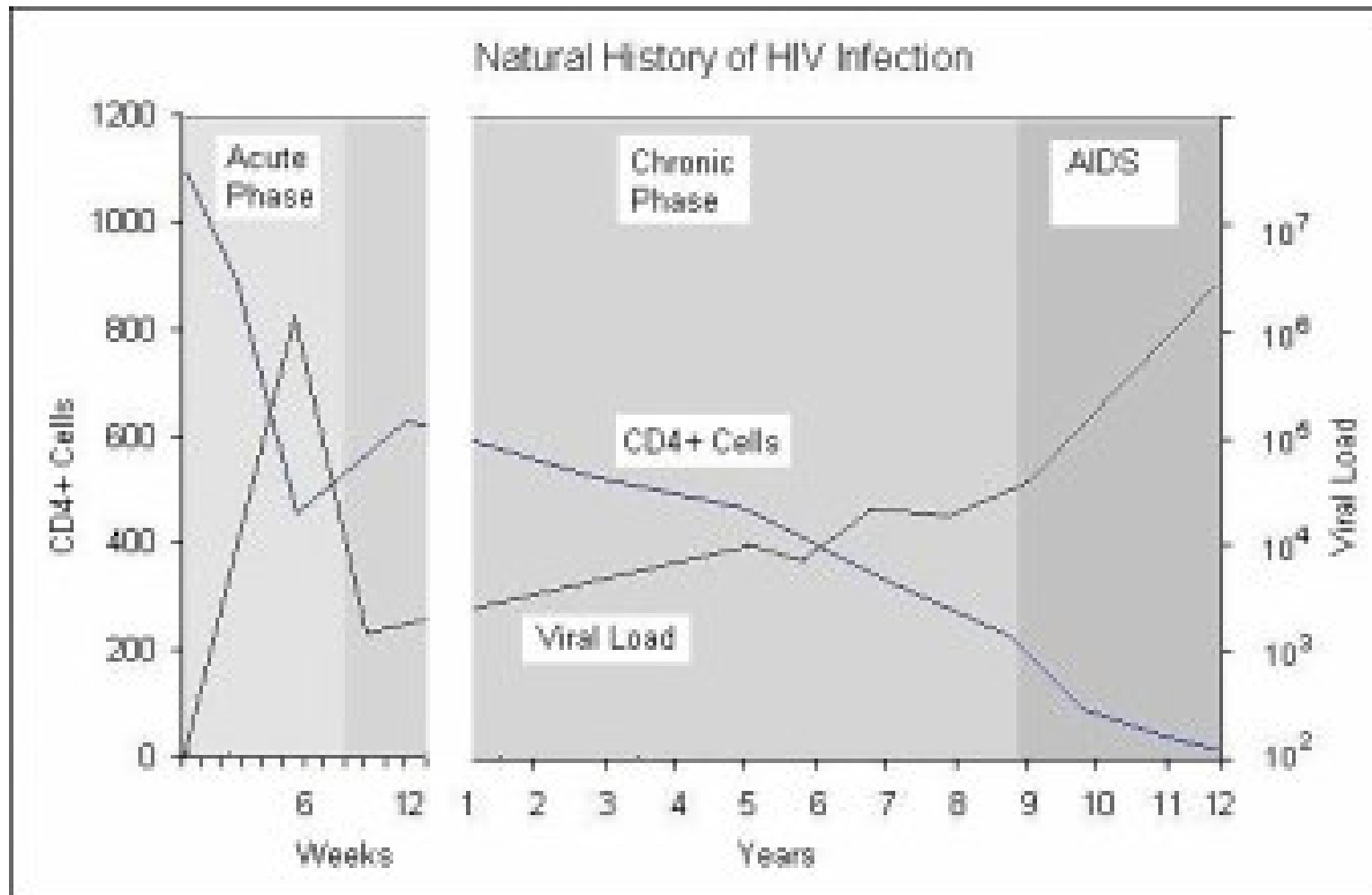


Fig. HIV Medicine 2006, Flying publisher



Introduction

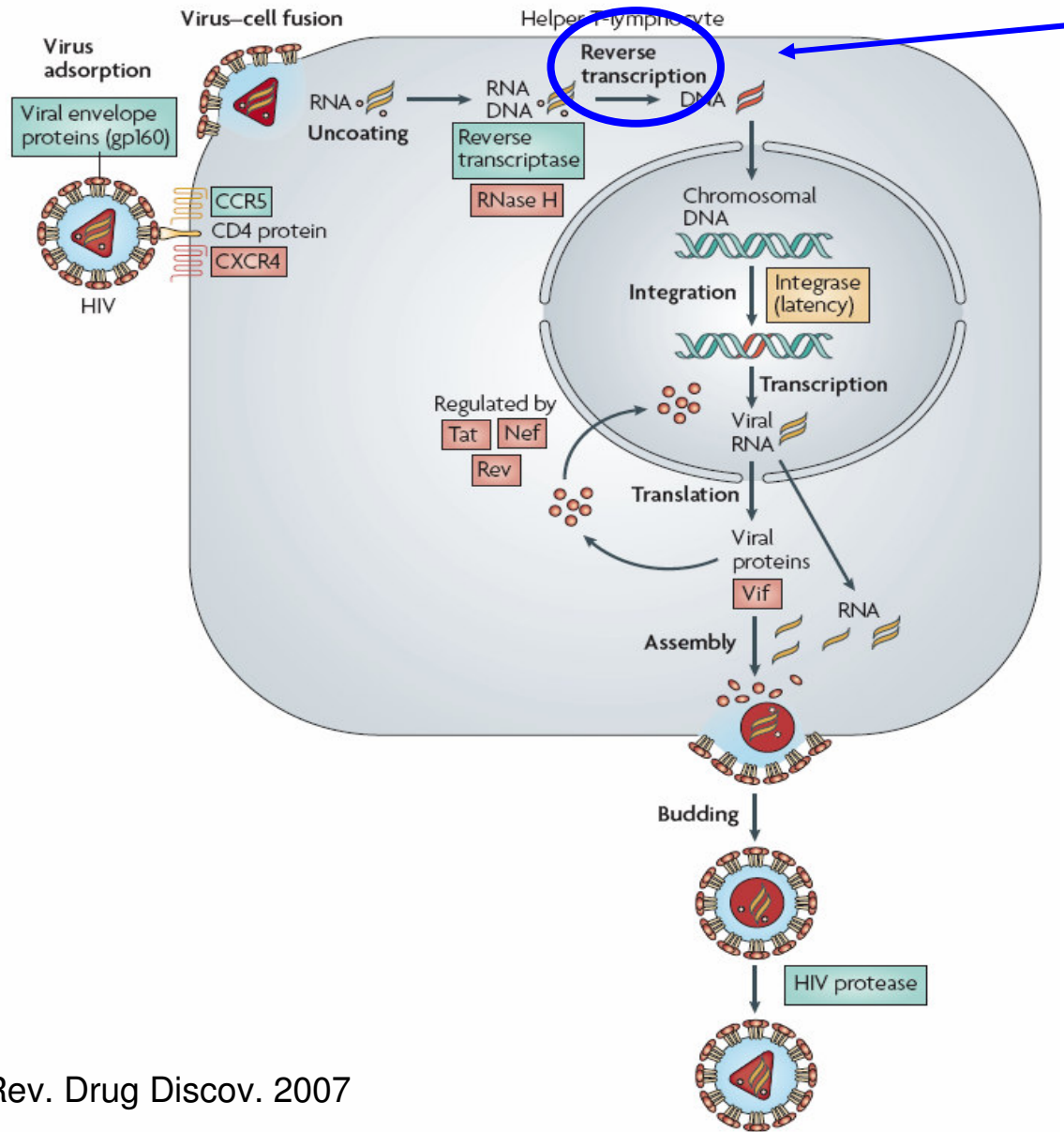
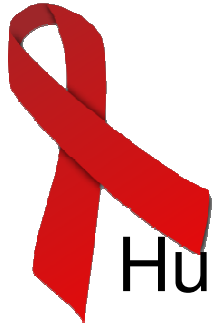


Fig Flexner, Nat. Rev. Drug Discov. 2007



Introduction

Human Immunodeficiency Virus (HIV-1) infection:

- Fast-progressors (FP) (< 2 years)
- Slow-progressors (SP)
- Non-progressors (NP) (>15 year)

Irrespective of type of transmission/donor

Non-progression is result of

- Virusrelated factors
- Hostrelated factors (eg. $\Delta 32$ deletion in CCR5 coreceptor gene)

Most studies involve NP groups → difficulties:

- Diversity between hosts
- Diversity between infecting viruses



Introduction

HIV-1 shows significant genetic diversity

- Between different hosts
- Within the same individual



- Primary infection with homogenous population
- From then on: evolution to heterogenous population of quasispecies to escape from pressures like immune system or antiretroviral drugs
- Most variable region: Env

Relation between variability & disease progression is controversial:

Less genetic diversity/viral evolution linked with **slower** progression

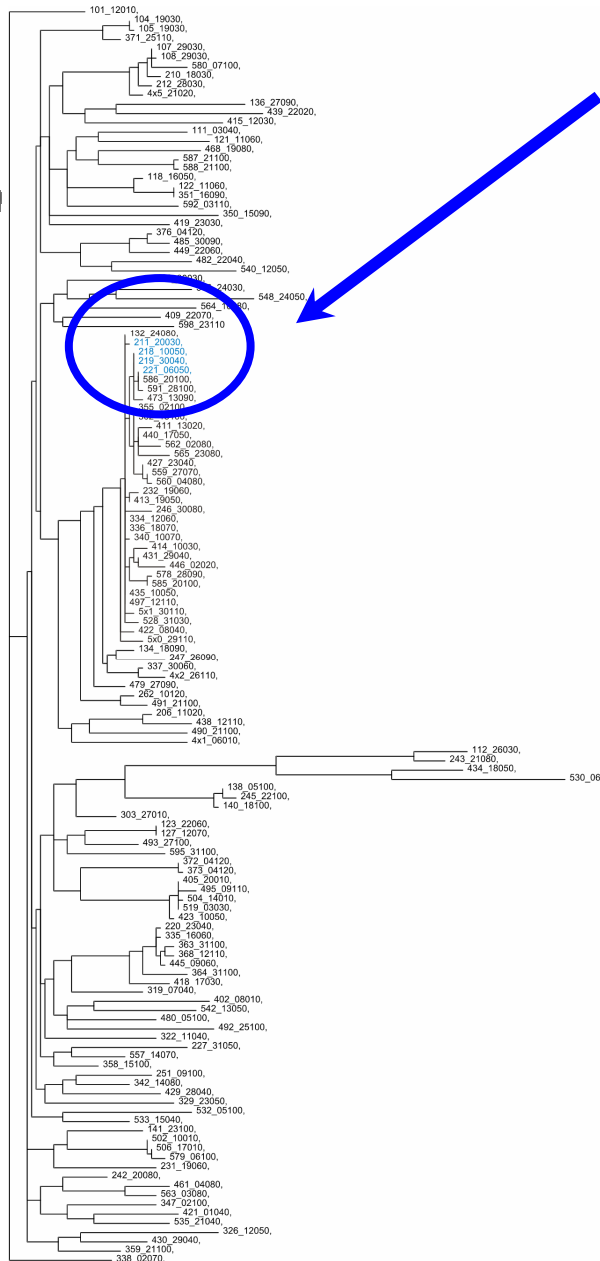
vs.

More genetic diversity/viral evolution linked with **slower** progression



Set up of this study

- ARC Ghent: More than 700 patients being followed
 - CD4⁺ en CD8⁺ T cell counts
 - Viral load
 - Sequence analysis of PR and RT (*pol* region)
- Phylogenetic analysis of *pol* region reveals relationship between viruses (Hue et al. Aids, 2004; Brenner et al., J.Infect. Dis. 2007)
 - clusters of patients, infected with closely related viruses
 - Selection of 4 patients



Phylogenetic tree constructed using heuristic maximum likelihood search



4 patients infected with almost identical virus (3 transmissions were epidemiologically confirmed)

Characteristics:

- Patients show significant differences in disease progression
- Primary infection with almost identical virus (HIV subtype B) → ≈ viral specific factors
- 2 patients with $\Delta 32$ deletion

→ Follow-up study of disease progression, viral diversity and viral evolution in env region during 4 years



Materials & Methods

- DNA extraction from PBMCs of 4 individuals
- Single genome sequencing of env region using limiting dilution
- Phylogenetic analysis using Paup*v4.0b10
 - Alignment in BioEdit using clustalW with manual correction
 - Selection of best fitting nucleotide-substitution model according the AIC using Modeltest v3.7
 - Construction of phylogenetic trees using Paup*v4.10b10 for a heuristic maximum likelihood search. Bootstrap analysis was performed on 100 replicates.

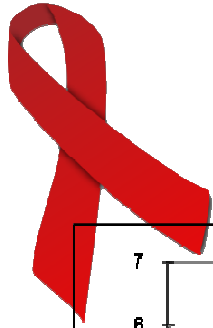


Results:

Confirmation of phylogenetic relationship between 4 individuals using env region of virus of primary infection

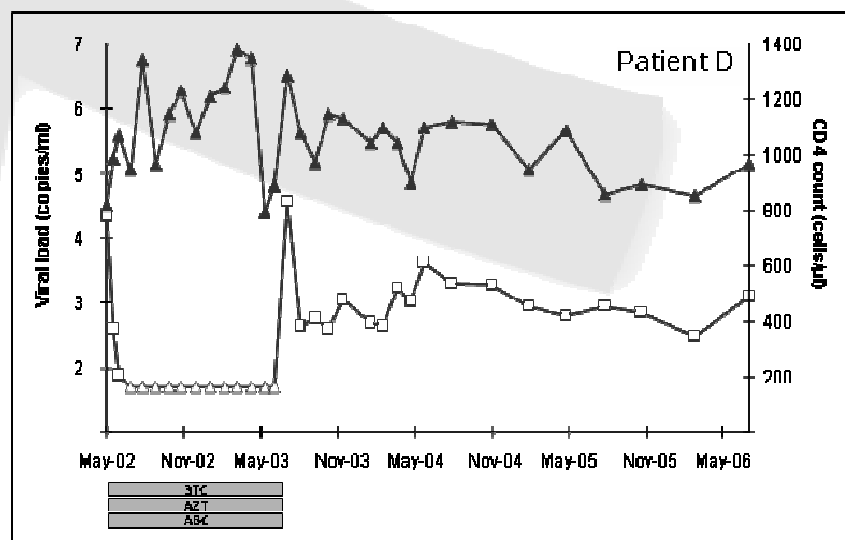
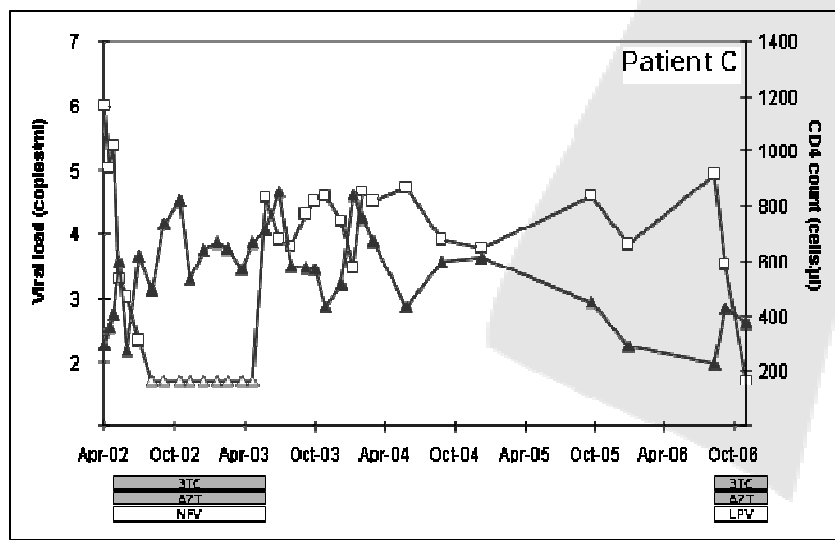
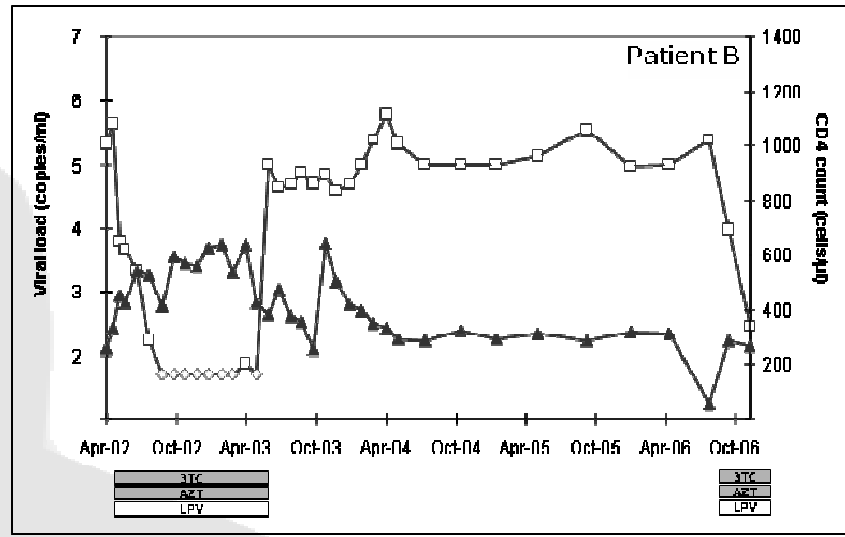
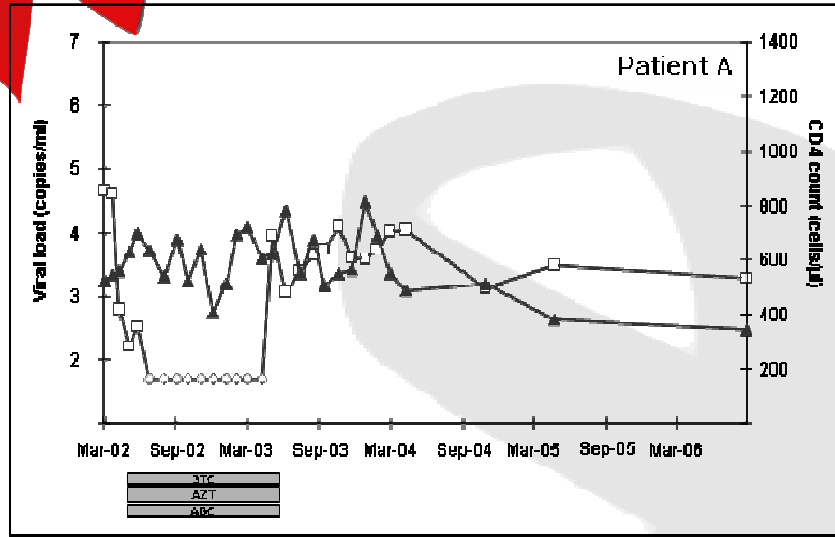


(outgroup is subtype A infected individual)



Results:

Follow-up of disease progression through viral load and CD4+ T Cell counts





Results:

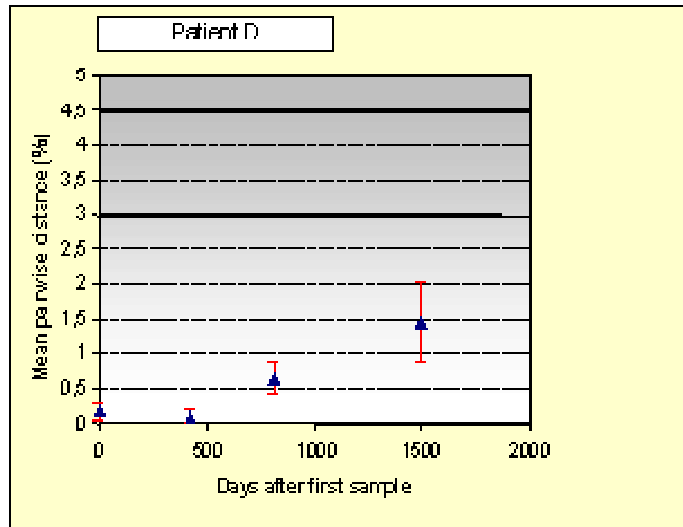
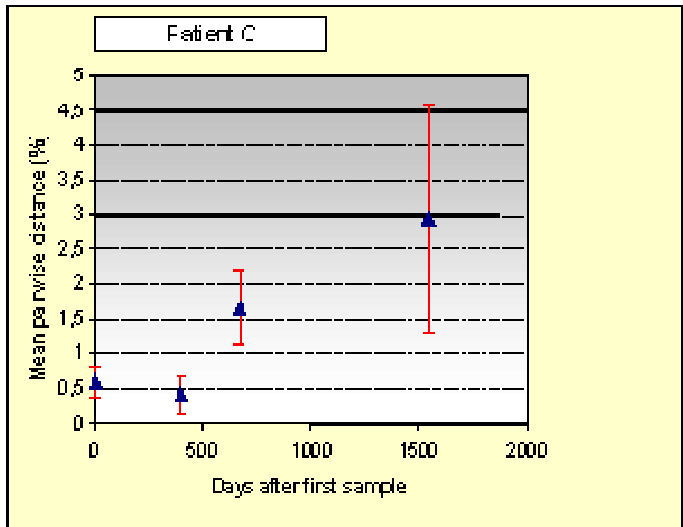
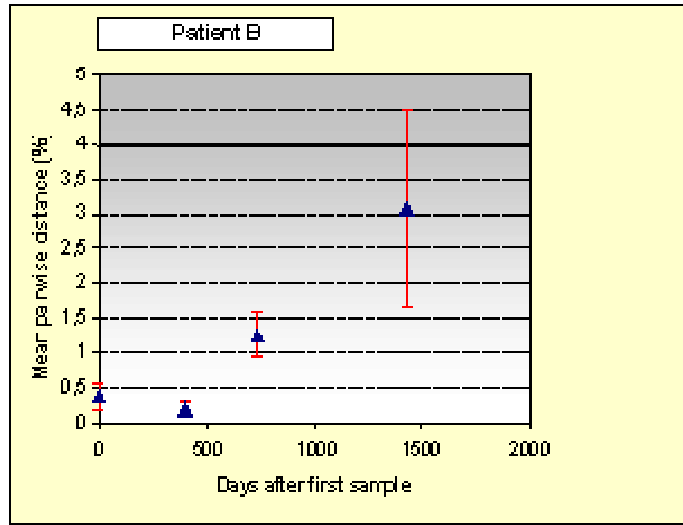
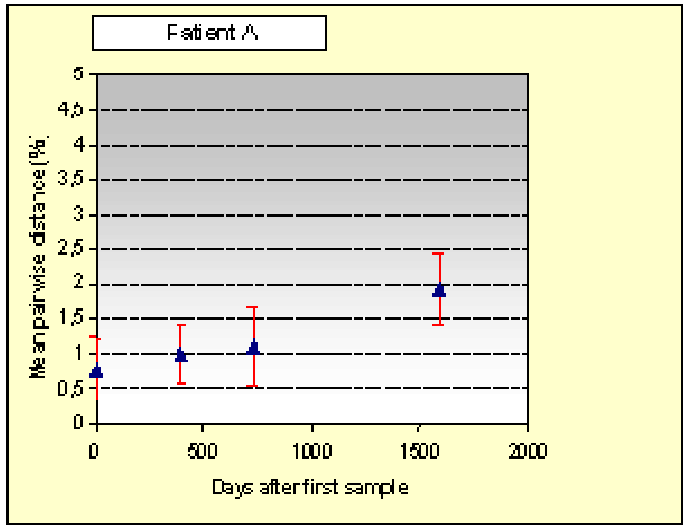
Analysis of inpatient sequence heterogeneity

Patient ID	CCR5 genotype	Collection date	Day	Viral load	CD4 count	N ^a	Mean nt difference ^b from consensus (range)	Mean fragment length ^c (range)	Mean pairwise distance ^d % (range)
A	Δ32/wt	04/10/02	0	39000	523	14	4,2 (1-11)	1011 (1011-1011)	0,73 (0,00-1,70)
A		05/22/03	402	8820	621	12	5,8 (1-13)	1011 (1008-1011)	0,92 (0,00-1,81)
A		04/28/04	738	10700	486	13	9,7(0-17)	1007 (993-1011)	1,11 (0,10-2,68)
A		09/13/06	1593	1960	345	10	37,4 (16-91)	1016 (993-1062)	2,21 (1,01-3,90)
B	wt/wt	05/10/02	0	424000	259	12	2,2 (0-5)	1011 (1011-1011)	0,34 (0,00-0,80)
B		06/20/03	400	>100000	385	10	2,4 (2-4)	1011 (1011-1011)	0,17 (0,00-0,40)
B		11/17/03	547	68300	646	10	5,8 (2-9)	1006 (999-1011)	0,70 (0,10-1,40)
B		05/26/04	736	210000	294	12	12,9 (1-20)	1009 (996-1014)	1,18 (0,20-2,02)
B		05/03/06	1433	>100000	315	10	74,8 (51-83)	1045 (1026-1053)	3,47 (0,20-6,55)
C	wt/wt	05/14/02	0	104000	361	13	3,5 (1-7)	1009 (1008-1011)	0,56 (0,20-1,30)
C		06/23/03	399	36100	716	10	3,9 (2-9)	1011 (1011-1011)	0,38 (0,00-1,00)
C		11/28/03	554	39300	437	14	8,8 (2-13)	1011 (1008-1011)	0,88 (0,00-1,71)
C		03/31/04	677	31800	675	13	29,4 (8-47)	1025 (1011-1044)	1,55 (0,69-2,75)
C		09/05/06	1551	85100	227	13	87,7 (75-107)	1044 (1032-1065)	2,95 (0,48-6,37)
D	Δ32/wt	05/06/02	0	21700	820	17	1,0 (0-3)	1011 (1011-1011)	0,17 (0,00-0,60)
D		07/07/03	421	36100	1290	11	0,6 (0-2)	1011 (1011-1011)	0,11 (0,00-0,30)
D		03/26/04	680	1710	1040	16	3,4 (0-8)	1011 (1011-1011)	0,51 (0,00-1,10)
D		08/02/04	806	2050	1120	11	7,2 (1-10)	1011 (1011-1014)	0,59 (0,20-1,20)
D		07/06/06	1500	1240	969	18	12,1 (0-25)	1011 (1011-1011)	1,37 (0,00-2,71)



Results:

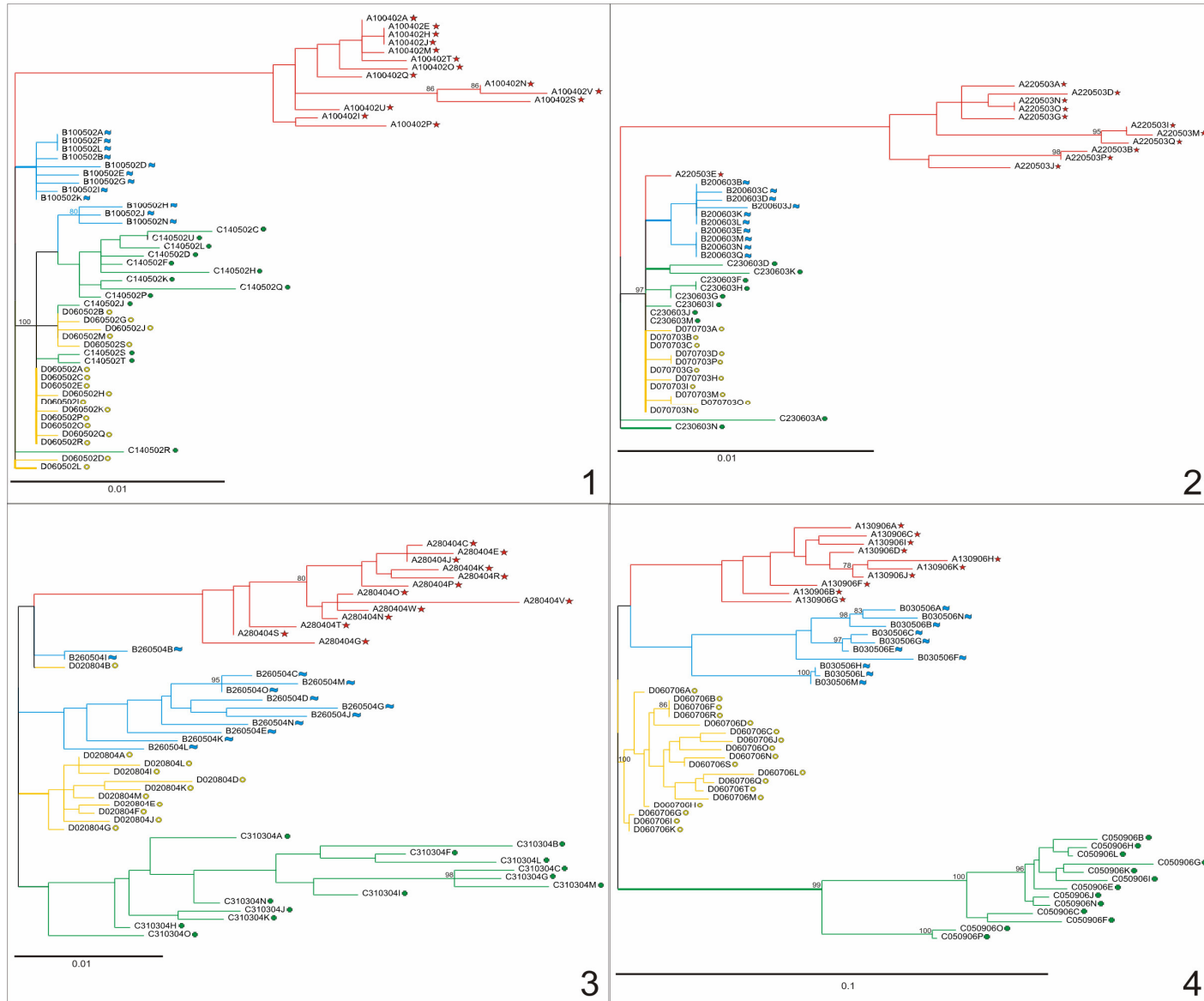
Analysis of mean pairwise genetic distance





Results:

Analysis of viral evolution





Conclusions

- Phylogenetic analysis of viral sequences obtained from 4 individuals infected with an almost identical HIV-1 virus allows us to study
 - intra-patient viral diversity
 - Intra-patient viral evolution
 - Inter-patient differences in viral evolution

Trough time

- Nearly identical virus shows different genetic evolutionary rates in different hosts
- More genetic diversity seems related to faster disease progression
- Individuals with $\delta 32$ -deletion show lower evolution rate and slower disease progression compared to wt individuals