

Evidence of frequent HIV-1 superinfection in a cohort of Kenyan women

Bhavna Chohan
and
Anne Piantadosi

Julie Overbaugh's Laboratory

Background

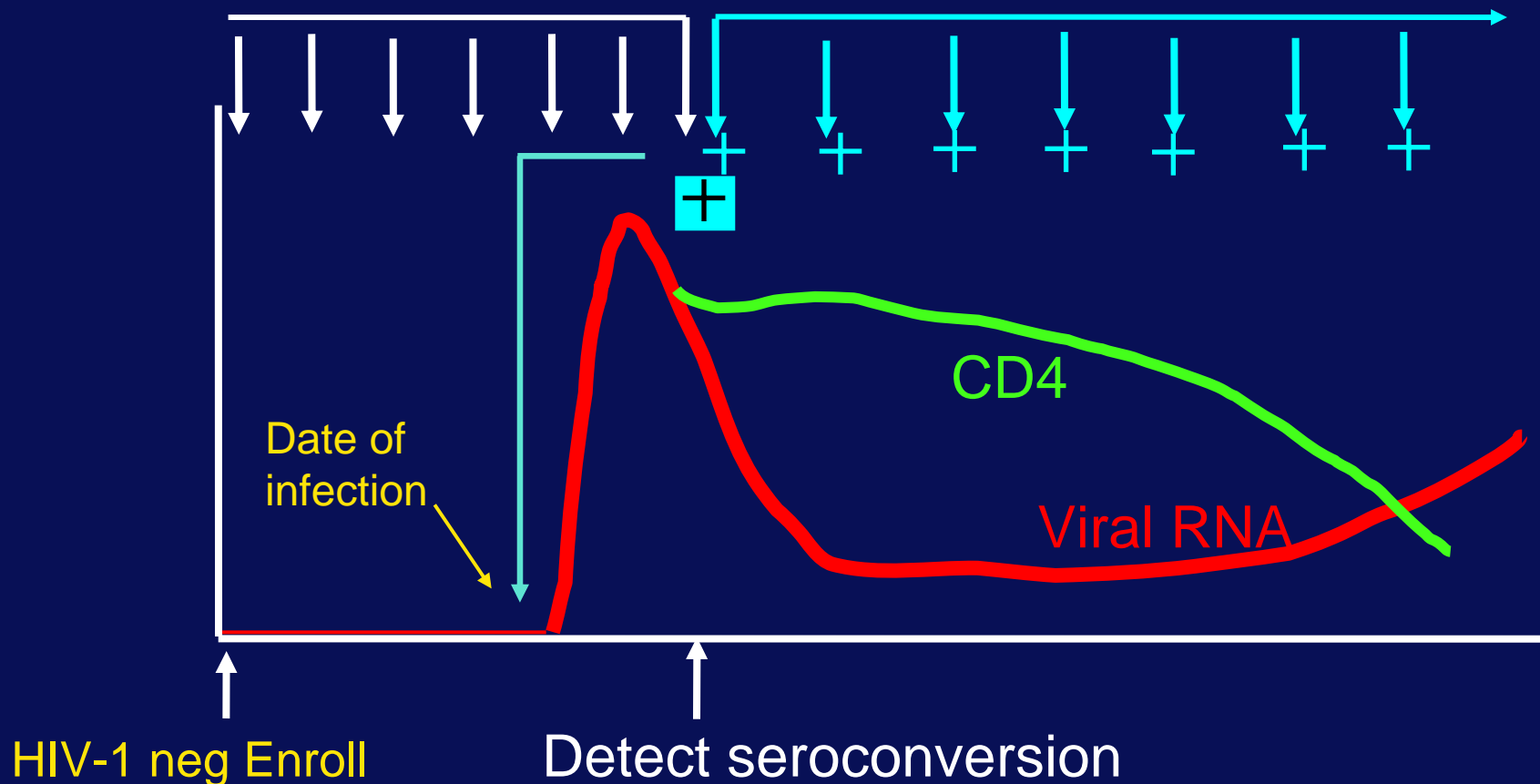
- More than 20 cases of HIV-1 superinfection have been reported:
 - more commonly with viruses that are distantly related (intersubtype)
 - few intrasubtype superinfection cases (mainly subtype B)
- Initial population-based studies measuring incidence of superinfection, focused on cases with limited follow-up:
 - superinfection was detected within ~ first year of initial infection, at an incidence approaching that of first infections. (Chohan B, et al., JV, 2005 and Smith DM, et al., JAMA, 2004).

The rationale of doing a larger study of the incidence of HIV-1 superinfection

- To determine how commonly HIV-1 superinfection occurs beyond one year of initial infection.
- To identify the frequency of superinfection with more closely related viruses (besides non-subtype B)
 - especially in heterosexual transmission.

Samples obtained from a prospective cohort of high risk women - Mombasa, Kenya (1993 - 2007)

Time of infection is determined by both serology and RNA testing



~ 1800 HIV-1 negative women enrolled with 300 seroconversions.
HIV-1 seroincidence of ~ 8.3 cases/100 py

Characteristics of Mombasa FSW cohort

- These women continue to be exposed to new strains of HIV-1.

They have an average of 1-2 sex partners per week, which is less than many sex worker cohorts.

- Long term follow-up, in some cases over a decade post initial infection.

- Three subtypes are common in this region:

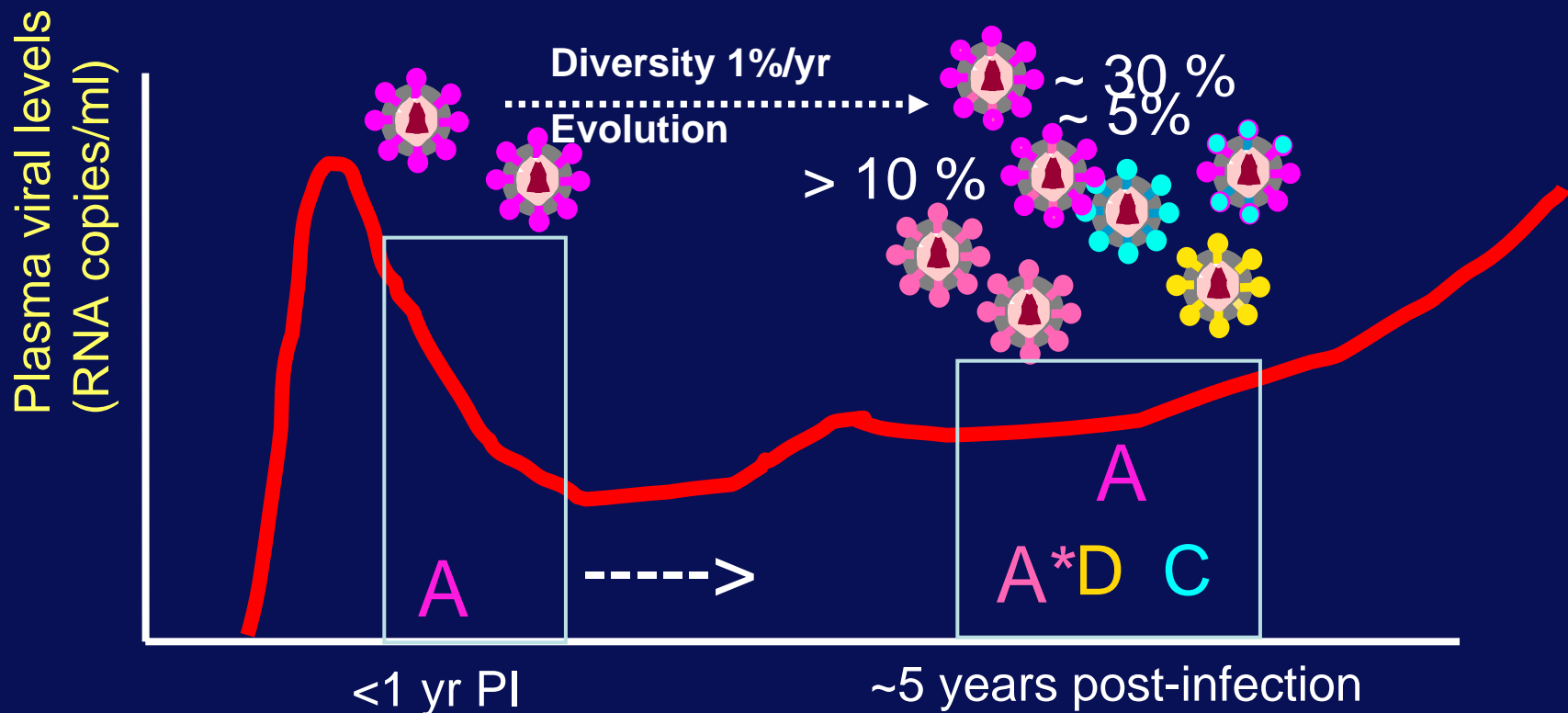
Subtype A ~ 80%

Subtype D ~12%

Subtype C ~ 7%

Study Design

- Analyzed 36 women within one year of initial infection who were infected with the most prevalent subtype (A) in Kenya
- With at least 5 yr follow-up and were ARV naïve - examine sequences at ~ 5 years PI



Methods and Results

- Phylogenetic analysis (maximum likelihood) and analyses divergence of viral sequences obtained from 36 women:
 - based on partial viral *gag* (670bp) and *envelope* (1.2kb) sequences,
 - obtained at two time-points;
 - early time point: range 17 - 338 DPI (median 111 DPI),
 - chronic infection: range 3.6 - 7.2 yrs (median 5.2 yrs PI)
- **Suggests 7 possible cases of superinfection**

Phylogenetic tree (ML) of *env* sequences from 36 women

Early and chronic viral sequences:

- cluster together suggesting single infection in most subjects;
- separate clustering suggests possible superinfection cases.

env



■ Case 4

■ Case 5

■ Case 3

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

■ Case 1

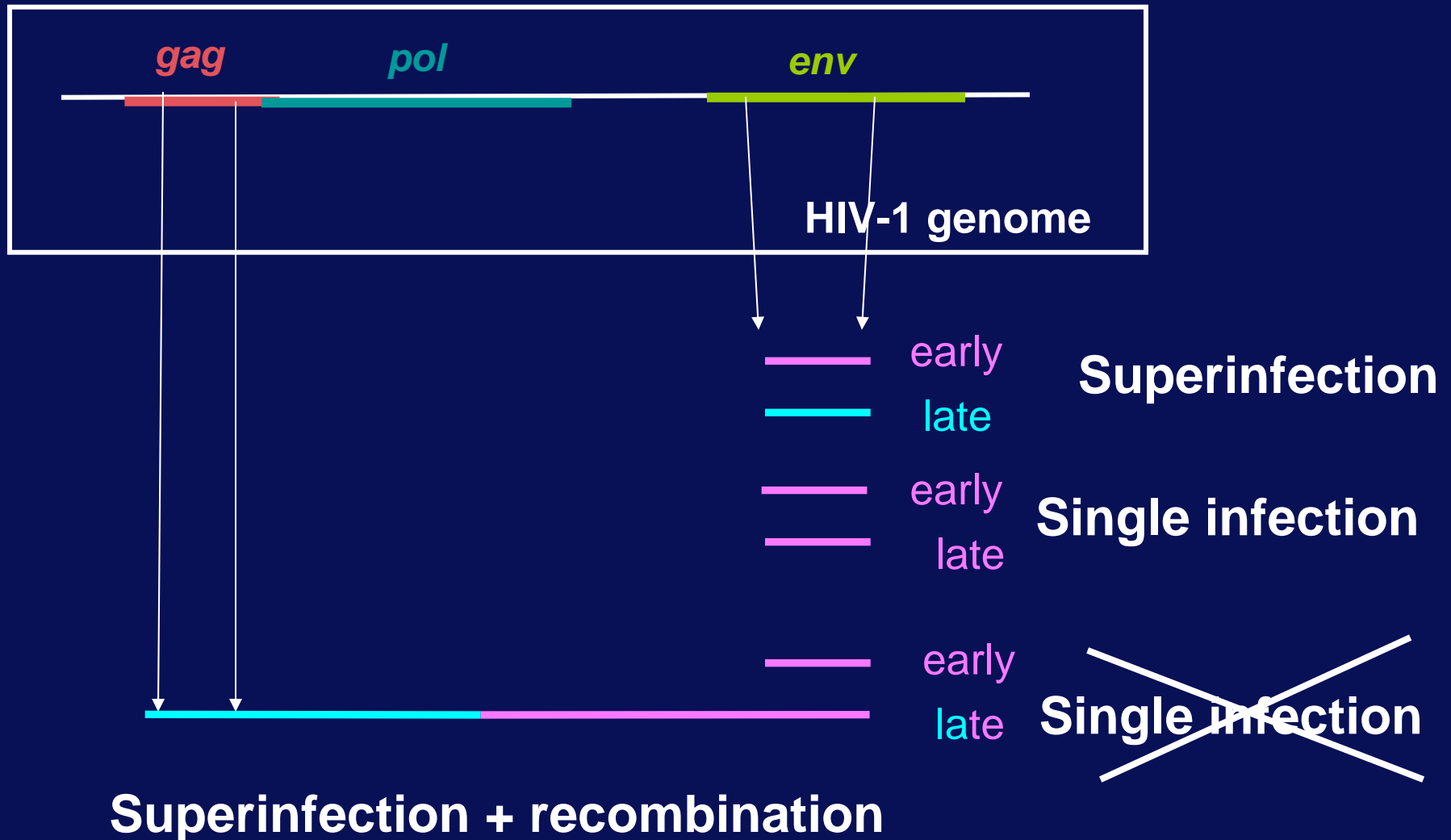


■ Case 2



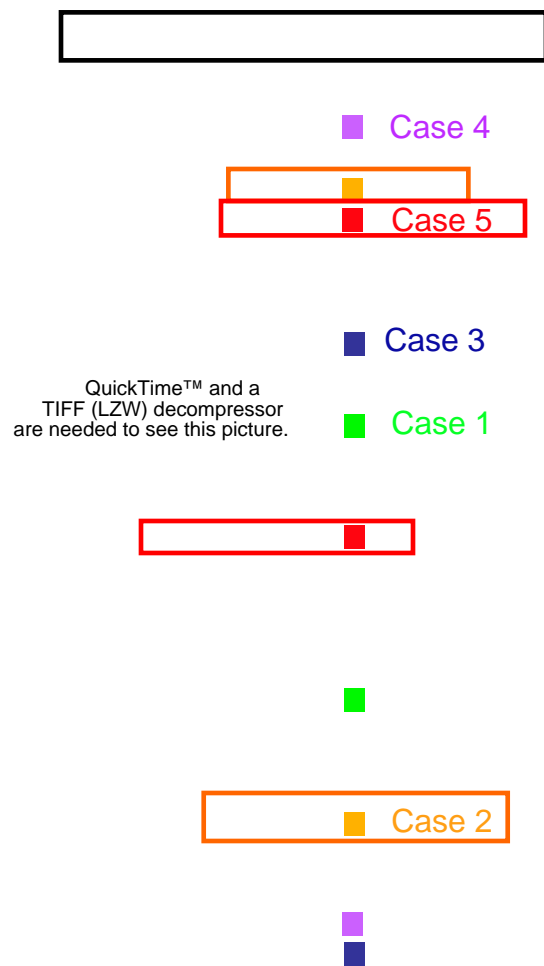
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Examining two regions of the genome may increase the detection of superinfection, because recombination could mask cases



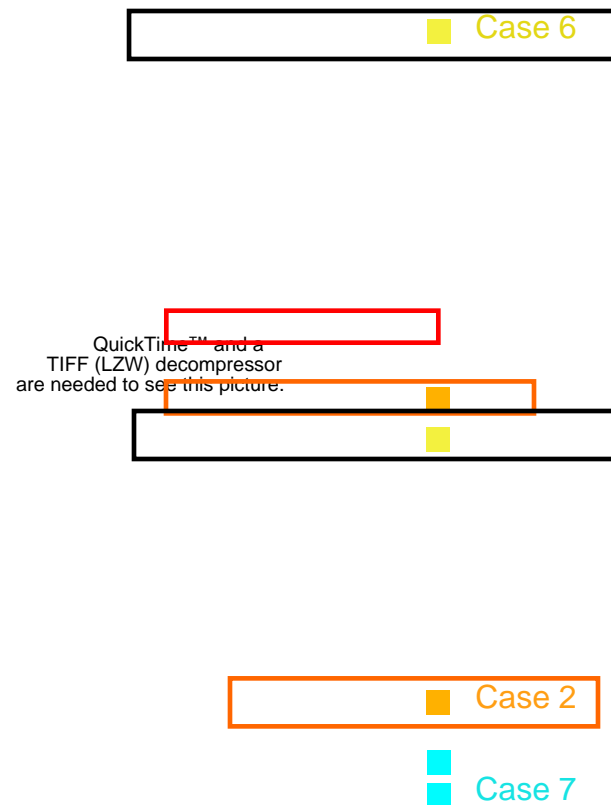
The 7 possible superinfection cases were detected in either env and/or gag sequences

env



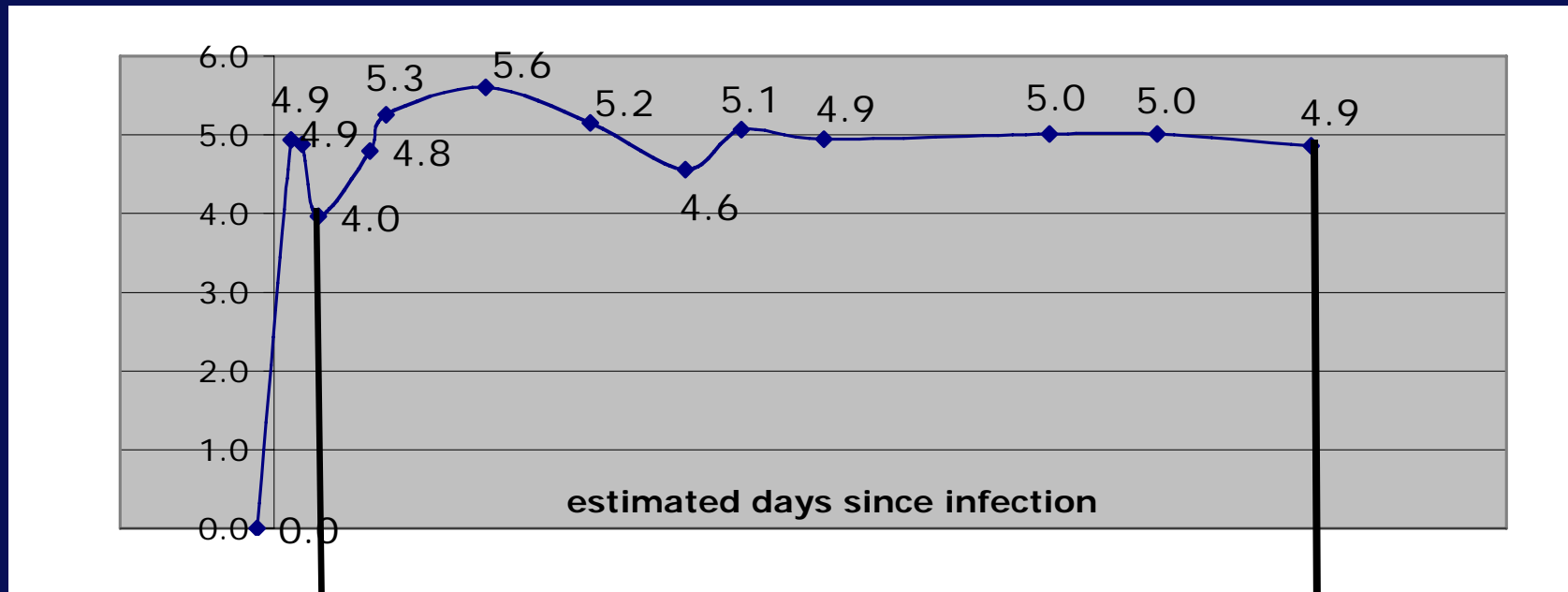
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gag



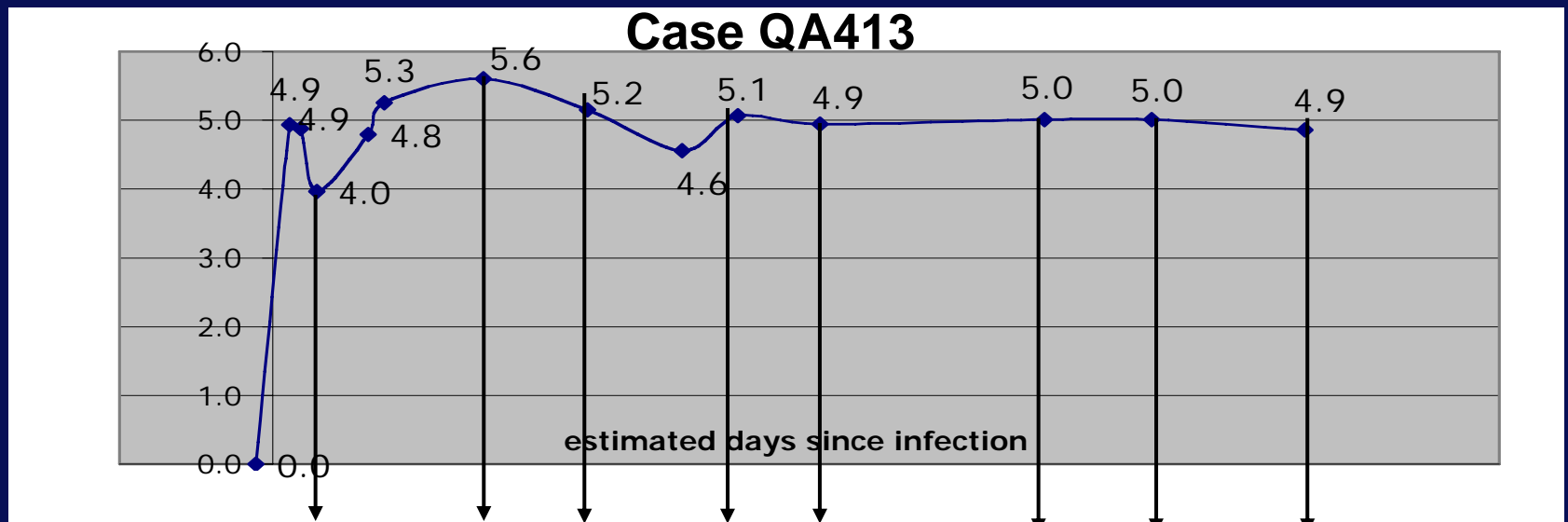
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One of possible case (QA413) of superinfection: Analysis of *env* sequences at initial and chronic time-points (DPI)



Days	57	1346
Sequence	A	A*
	+	+
	11	3
	-	+
		8

Estimate time of superinfection: analysis of sequences and Allele-specific PCR from sequential time-points



Days	57	275	411	607	714	1007	1146	1346
sequence								
A	+	+	+	+	+	+	+	+
A*	—	—	—	—	—	+	+	+
SSP-PCR								
PBMC								
A	+	+	+	+	+	+	+	+
A*	—	—	—	—	—	+	+	+

Superinfection between 714 - 1007 days PI (midpt = 2.4 yrs PI)

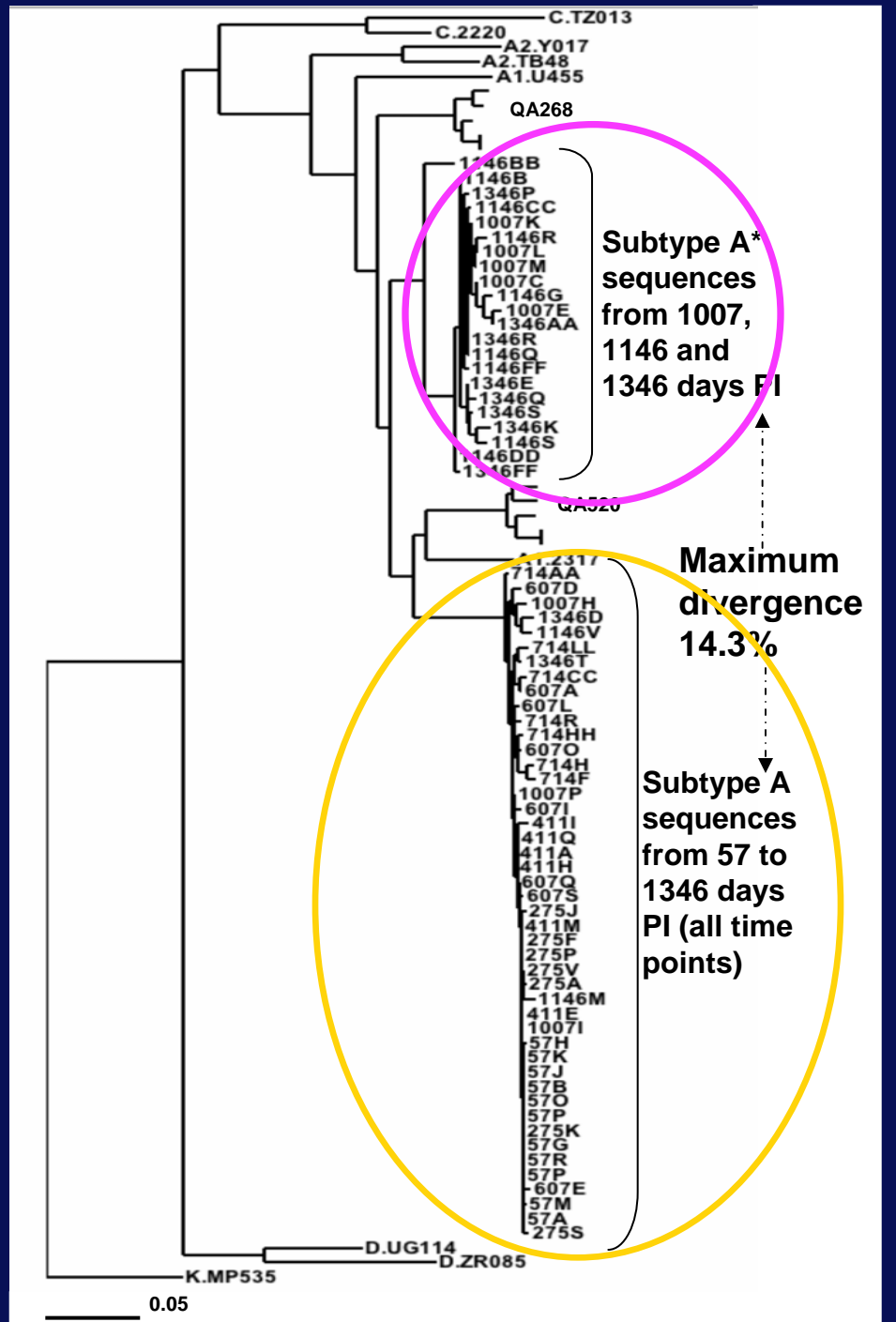
Phylogenetic tree (ML) of *env* sequences from all time points: case QA413

Early and chronic viral sequences:

- from all time-points cluster together with initial subtype A

Some viral sequences from 1007 days onwards:

- form a separate cluster of new subtype A (A*) viruses
- divergence of ~ 14% from initial subtype A sequence



Case of HIV-1 superinfection - QA413

- A case of **intrasubtype** superinfection
 - initially infected with subtype A and reinfected with another subtype A (A*).
- Superinfection occurred in the face of high viral load and during chronic infection ~ **2.4 yrs PI**
- After superinfection, both subtype A viruses **coexisted** together through 3.7 yrs PI.

Summary - Superinfection in 7 cases occurred at different times after initial infection

From the 36 women - 7 women identified as possible superinfection cases in *gag* and/or *env* sequences

ID	Estimated time to superinfection	Subtype (<i>env</i>) initial chronic		Subtype (<i>gag</i>) initial chronic		Type of reinfection
QA413	~ 2.4 yrs	A	A + A*	A	A	intrasubtype
QB685	~ 2.4 yrs	A	A	A	A*	intrasubtype
QB045	~ 5.1 yrs	A2	A2 + A1	D/A2	D/A2 + D/A1	intrasubtype
QB726	~ 2.4 yrs	A	A + A*	D	D + A1	intersubtype
QD022	~ 5.2 yrs	A	C	A	A	intersubtype
QB850	~ 2 months	A	A + A/D	A	A	intersubtype
QC885	~ 3.6 months	A	A	C	C + A/C	intersubtype

Conclusions (1)

- Incidence of HIV-1 superinfection is high in these high-risk women
 - the incidence of superinfection was ~ half the rate of initial incidence in cohort.
- Analyses of 2 regions of genome allowed detection of cases of superinfection - would have been missed if only one region were analyzed
 - may suggest even more cases would be detected if the whole genome was analyzed at multiple times PI.
- Superinfection may occur at different times after the initial infection
 - superinfection detected within a range of 2 months - 5 years: superinfection is not limited to a 'window' after initial infection.

Conclusions (2)

- Superinfection is not limited to viral strains that are distantly related; it can occur with closely related viruses
 - 3 out of 7 cases were intrasubtype superinfection.
- Superinfection can occur in the face of high viral replication and is not limited to cases where the first virus is of low replication fitness
 - both viruses can exist at similar levels for several years.

Implications of HIV-1 superinfection

The high frequency of HIV-1 superinfection suggests that it may be common

- suggesting that immune responses generated to natural HIV-1 infection may not be protective.
- additional studies examining immune responses in population-based cases of superinfection may provide insights into immune correlates that do not provide protection in HIV-1 infected individuals.

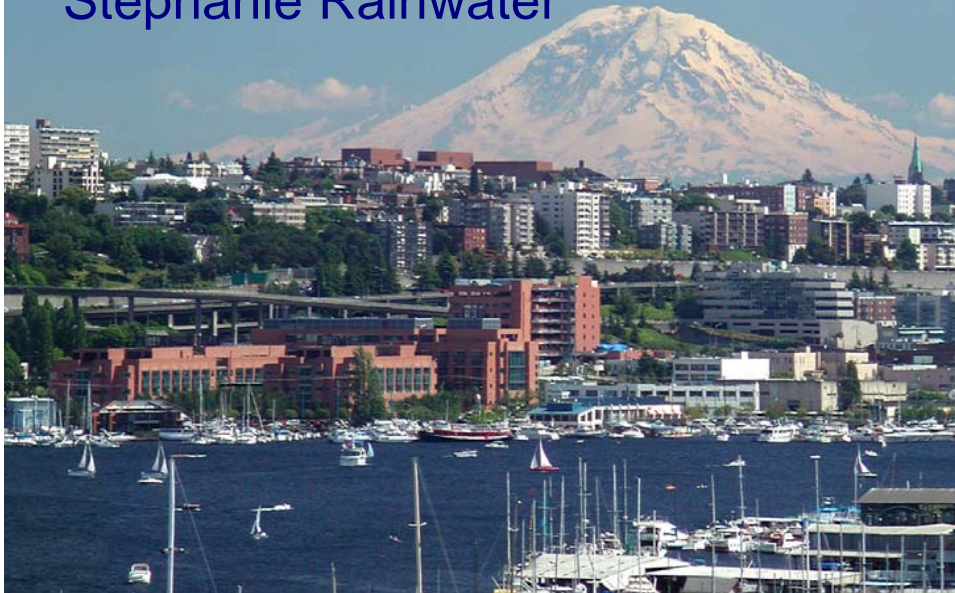
Overbaugh lab
Fred Hutch Center

Mombasa, Kenya
U Nairobi AIDS project

Julie Overbaugh

Anne Piantadosi

Stephanie Rainwater



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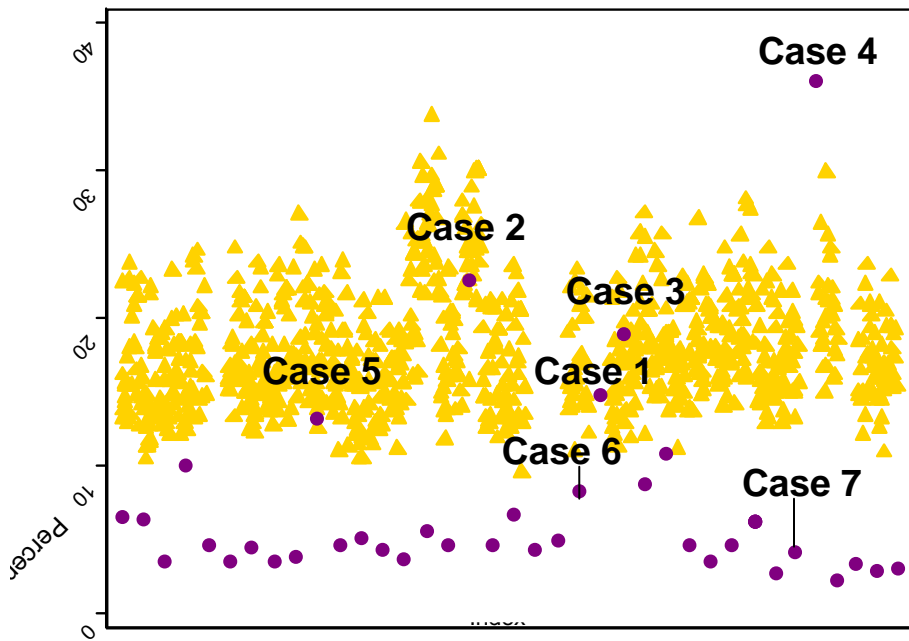
Ganjoni Clinic
Laboratory
All participants in study



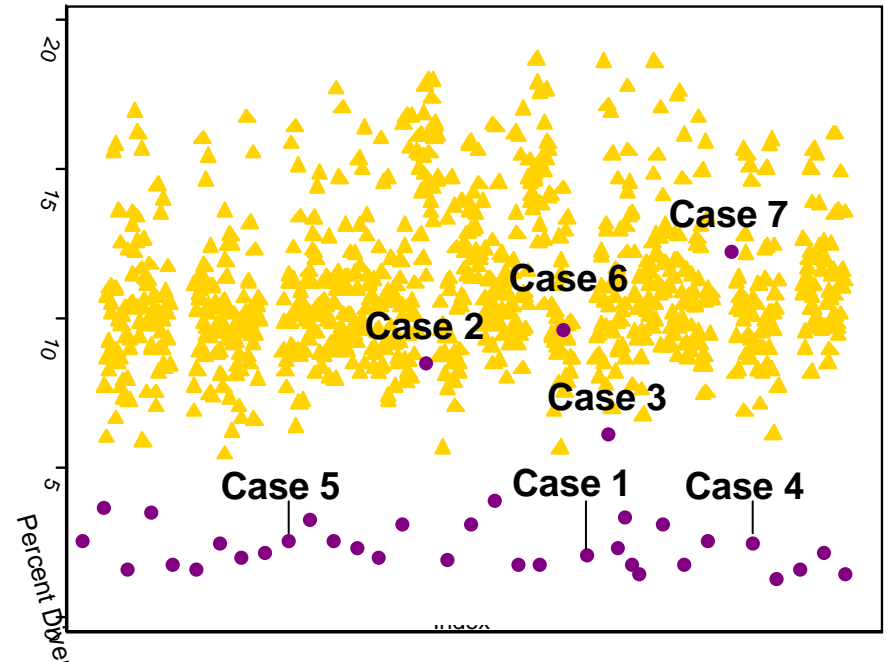


Divergence for each simulated superinfection

env



gag



Gold: simulated superinfections (subtype A), *env* = range 10.4 - 34.2%;
gag = range 5.4 - 17.1%

Purple: subjects

Strain-subtype specific PCR of PBMC DNA QA413

