Selection in HIV-1 gp41 in acute infection

AIDS Vaccine Conference

2008

Gama Bandawe

gp41

- gp120 and gp41 have functionally distinct but additive roles in HIV infection and pathogenesis (Meissner et al, 2005)

- gp120 mediates transmission via CD4 binding while gp41 is essential for post CD4 binding events including viral fusion and assembly (Lambele´et al 2006; Martin et al. 1996; Mobley et al 1999; Charloteaux et al 2006; Dey et al 2006)

- Post CD4 binding (and gp41 related) processes are among the most significant determinants of replicative capacity and pathogenic potential in any given strain (Hsu et al, 2003)

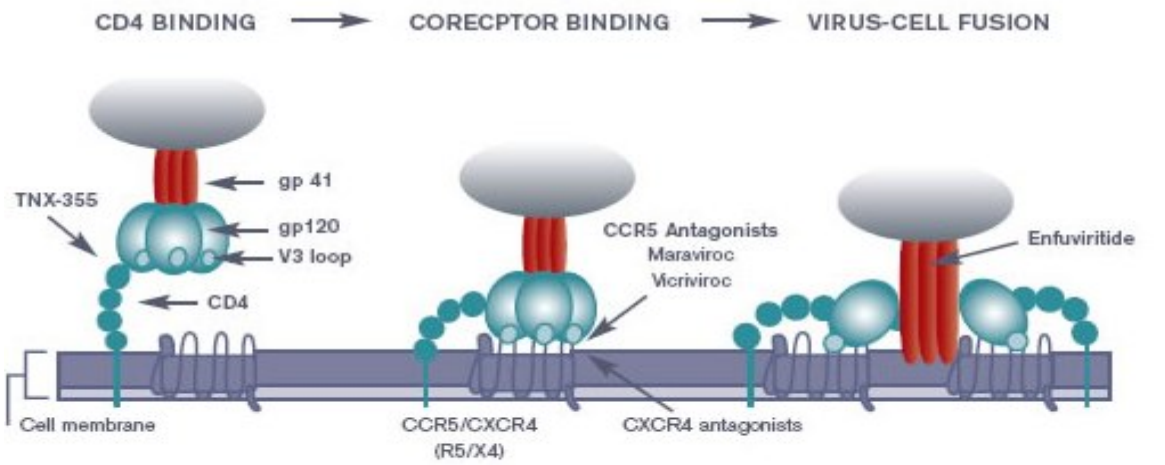
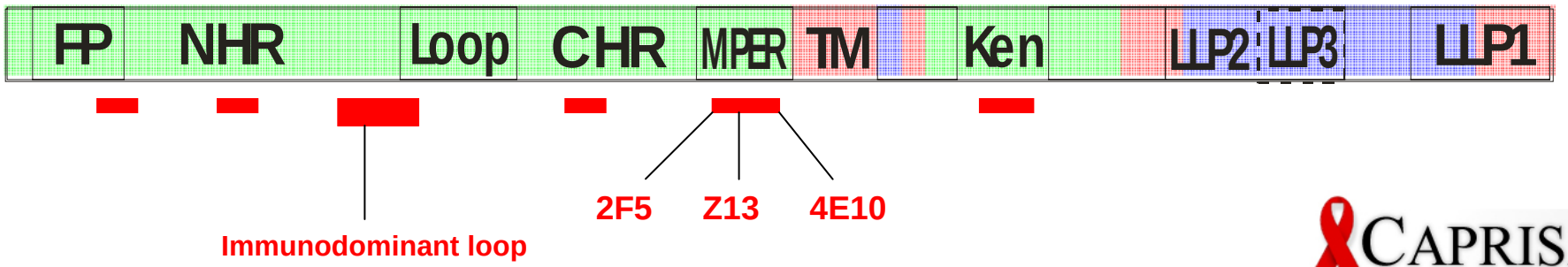


Figure adapted from Doms R. et al Genes Dev. 2000. 14:2677-2688

Gp41 in vaccines

- Gp41 is conserved relative to gp120 with fewer indels
- It is one of the earliest targets of the immune response
- Several regions of gp41 elicit Ab reactivity.
- Of the 6 known broadly neutralizing Abs 3 are found in gp41 (2F5, Z13 and 4E10)



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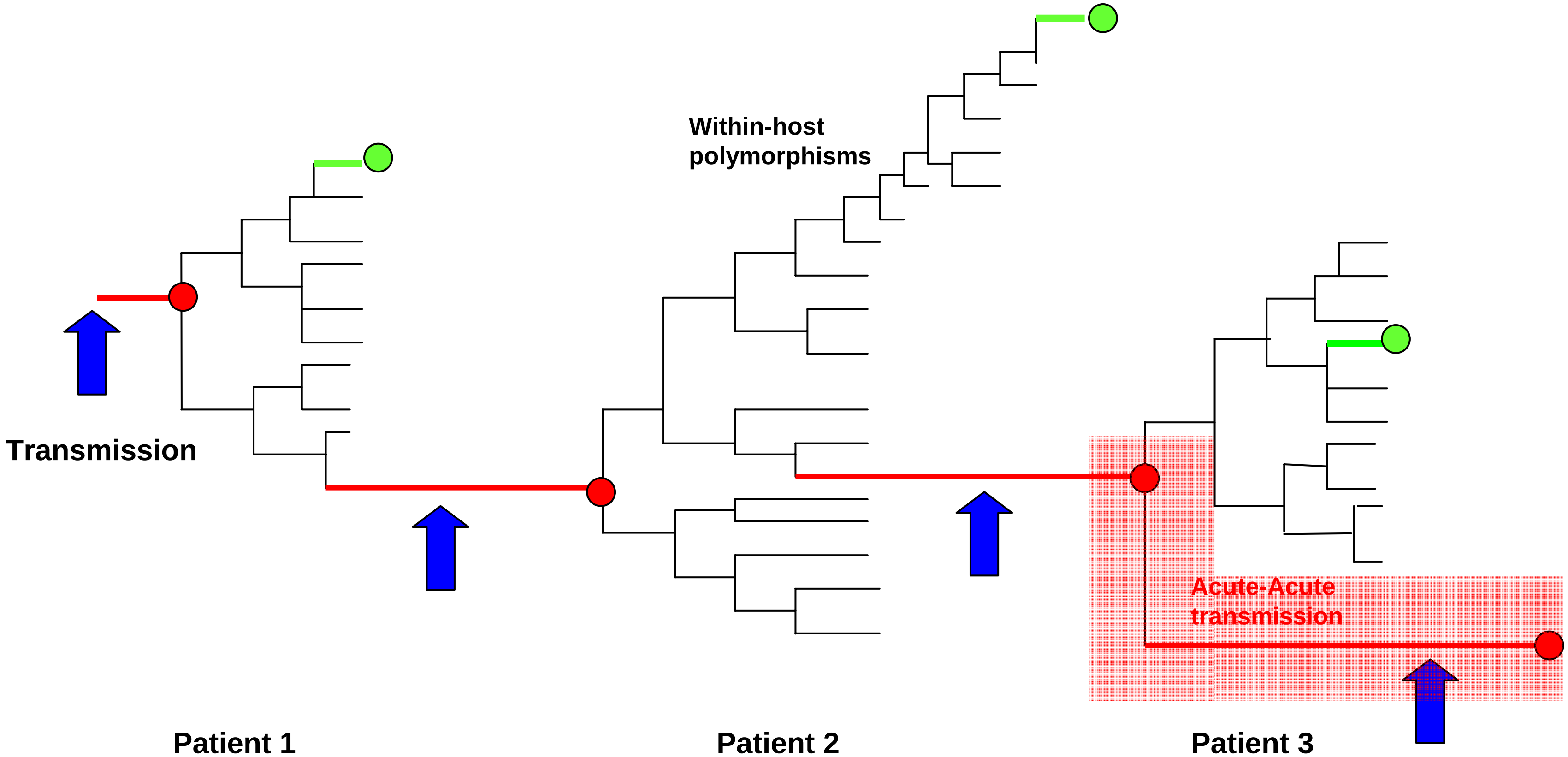
Acute infection, chronic infection and transmission



The HIV transmission chain comprises a repetitive series of

Selection signals differentiating intra-host and inter host adaptation

terminal branches

adaptations ... chronically infected patients

(Herbeck et al 2006)

Study aims

- **Is it possible to detect signals associated with transmission separate from host specific adaptation?**

- **Which sites are associated with this sort of adaptation?**

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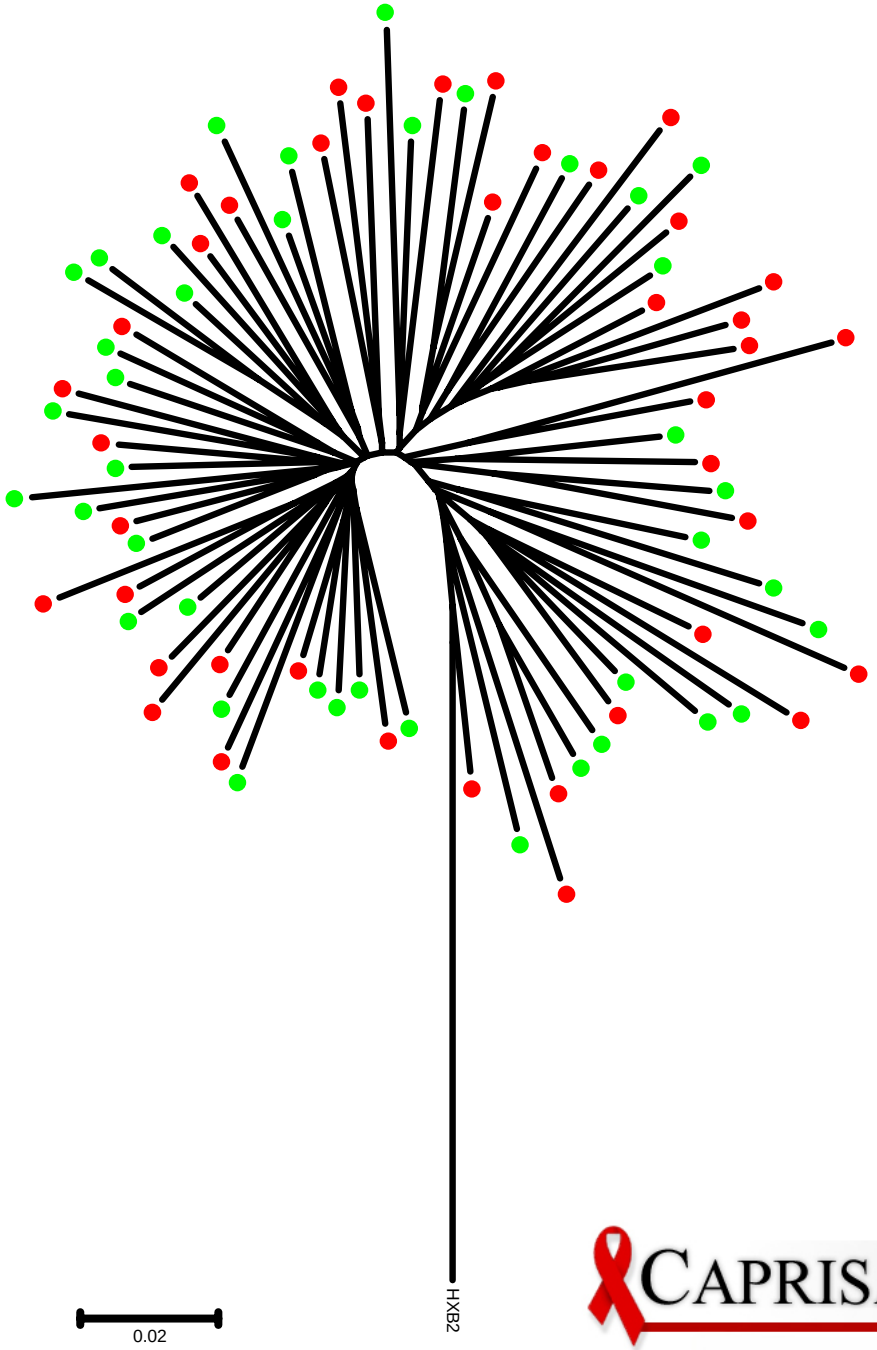
Subtype C datasets

40 CAPRISA enrolment gp41 sequences sampled at a mean 40 days post infection. This is the **Acute/early** Infection (**AI**) dataset

40 **chronic** HIV-1 subtype C sequences matched for gender and geographic location from the Kiepiela et al (2004) study. CD4 counts <200 and viral loads >200000 were excluded. This constituted the chronic infection (**CI**) dataset



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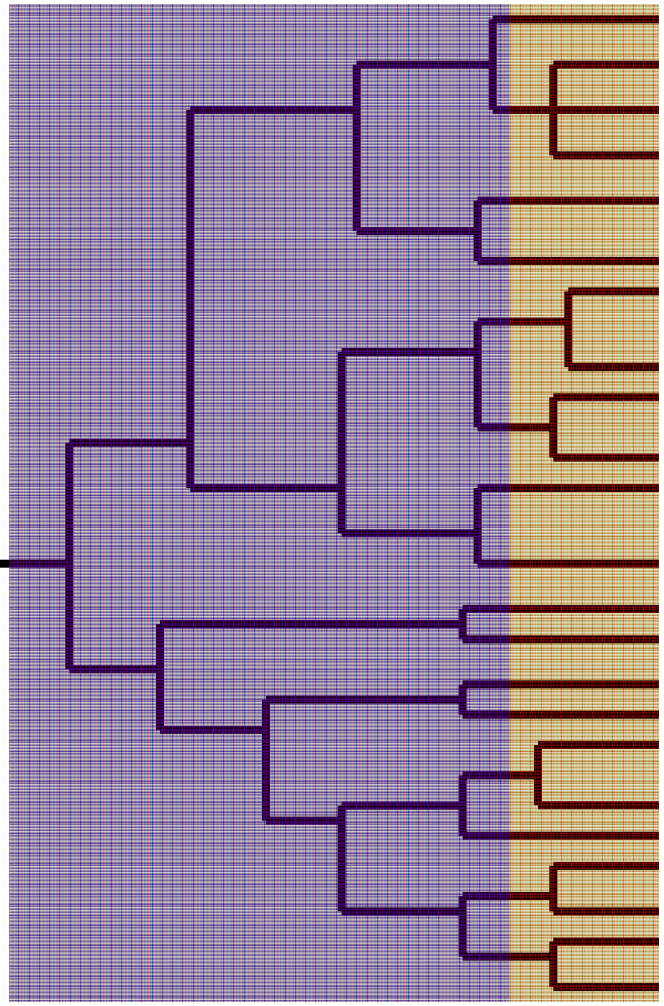
Methods of inference of selection



FEL

Direct counting of substitutions along phylogeny from ancestral state

Fitting **ML** substitution rates on a **site by site** basis

Uses **likelihood ratio tests**

No assumption of distribution of rates

Good for **large datasets**

Computationally intense

Lacks power with **small** or **low diversity** datasets

SLAC

This **removes** the effect of **recent** and possibly **transient**, within host substitutions (**on terminal branches**)

therefore detects **population level** signals of selection

All methods are able to accommodate recombination by creating separate trees between potential breakpoints

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DATAMONKEY
RAPID DETECTION OF POSITIVE SELECTIO
a Web-Server of the H Package
Welcome to the free public server for detecting signatures of positive and negative selection from coding sequence alignments using state-of-the-art statistical models. This service is brought to you by the viral evolution group at the Antiviral Research Center of the University of California, San Diego. The methods and software tools are developed and maintained by Sergei L Kosakovsky Pond, Simon Frost and Art Poon.
March 17th, 2008: Spidermonkey - a new tool for discovering evolutionary dependancies between sites in a coding alignment using Bayesian Graphical Models (BGMs) has been added to Datamonkey. The SpidermonkeyBGM analysis option is available for non-recombinant coding alignments of up to 150 sequences.
Datamonkey.org can help you answer the following questions (publications citing datamonkey.org) :
Which codon sites are under positive or negative selection?
Three different codon-based maximum likelihood methods, SLAC, FEL and REL, can be used estimate the dN/dS (also known as Ka/Ks or ω) ratio at every codon in the alignment. An exhaustive discussion of each approach can be found in the methodology paper. All methods can also take recombination into account. This is done by screening the sequences for recombination breakpoints, identifying non-recombinant regions GARD tool and allowing each to have its own phylogentic tree.
Is there evidence of selection in my alignment?
The PARRIS method, developed by Konrad Scheffler and colleagues, extends traditional codon-based likelihood ratio tests to detect if a proportion of sites in the alignment evolve with dN/dS>1. The method takes recombination and synonymous rate variation into account.
Which codon sites are under positive or negative selection at the population level?
The codon-based maximum likelihood FEL method can investigate whether sequences sampled from a population (e.g. viral sequences from different hosts) have been subject to selective pressure at the population level (i.e. along internal branches). A discussion of the method and its
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Test of the methods

- **Are the methods used reliable?**
- **How do sites detected by these methods compare to sites described previously?**

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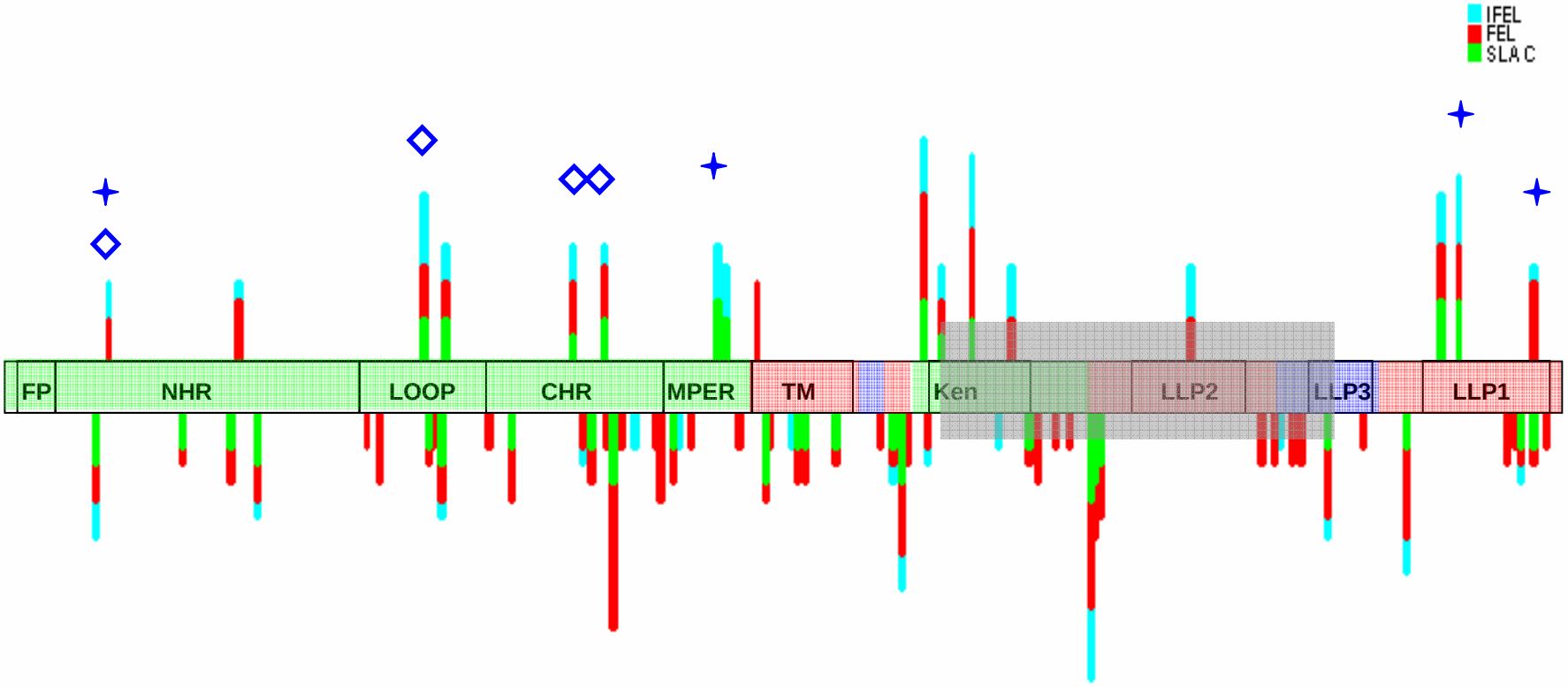

We used 8 datasets of about 40 sequences from different subtypes and CRFs

Purifying selection was very pervasive and often gave stronger signal

7 out of 12 sites we identified were previously identified as sites probably under positive selection in multiple subtypes

Rev exon 2 overlapping reading frame

external

membrane

internal

Choisy et al 2003

Travers et al 2005

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Hypothesis

- Given the largely overlapping phylogenetic history, the two subtype C datasets should share more selection signals that other subtypes

- sites where the selection signal is strongest in the terminal branches (intra-host adaptation) should be, less conserved between the AI and CI datasets than those detectable throughout the tree

- Differential selection detected in terminal branches of AI and CI datasets should be indicative of different selection pressures related to these stages.

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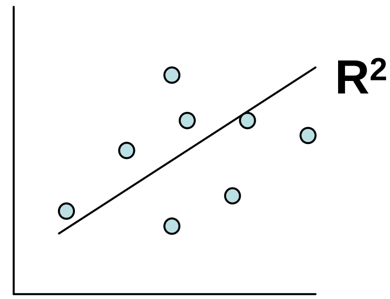
Normalized dN/dS

All signals

AI	CI	Sub A
0.0024	0.0006	0.0051
0.0006	0.0071	0.0468
0.0071	0.0034	0.0321
0.0034	0.0294	0.0006
0.0294	0.0051	0.0071
0.0426	0.0468	0.0034
0.0122	0.0321	0.0294

Subset of signals

AI	CI	Sub A
0.0024	0.0189	0.0013
0.0006	0.0376	0.0291
0.0071	0.0051	0.0059
0.0034	0.0468	0.0107
0.0294	0.0321	0.0084



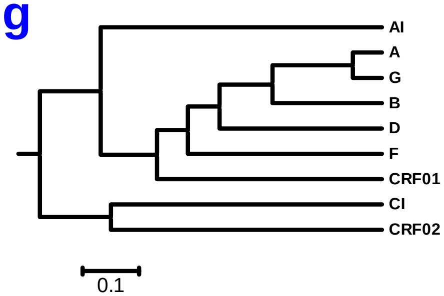
Regression analysis

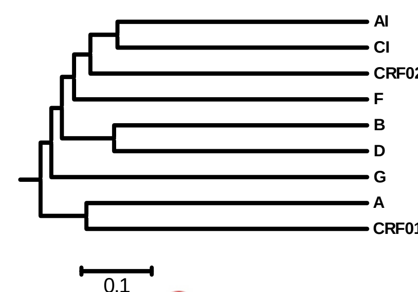
(1-R)

	AI	CI	Sub A	Sub B
AI	0			
CI	0.0006	0		
Sub A	0.0071	0.0051	0	
Sub B	0.0034	0.0468	0.0107	0
Sub D	0.0294	0.0321	0.0084	0.0321

Matrix or R-1 values

UPGMA or NJ clustering algorithm





For all 9 datasets we took dN/dS ratios from sites with any significant evidence (p<0.05) of either positive or negative selection

We then did the pair-wise regression analyses to determine (1-R values) between all datasets

We used UPGMA and neighbour-joining algorithms to visualise relatedness of selection signals in dendrograms

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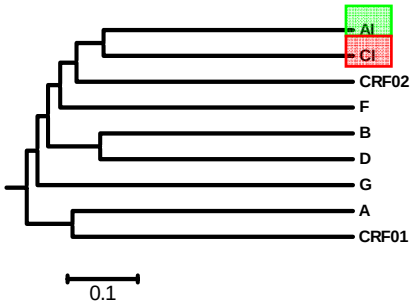
UPGMA dendograms of regression analysis of all selection signals

Purifying selection signals

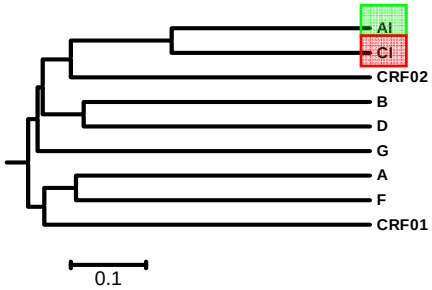
FEL

a)



IFEL

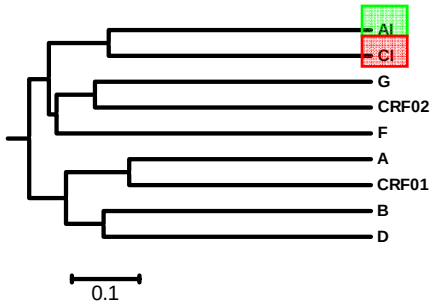
c)



Diversifying selection signals

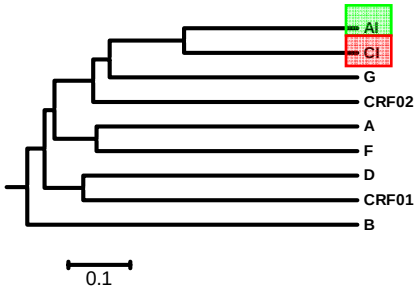
FEL

b)



IFEL

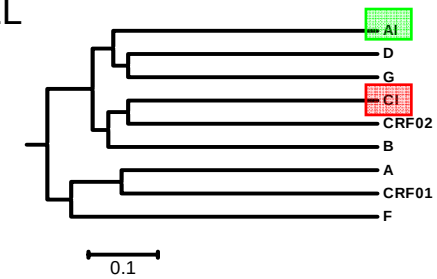
d)



Differences detected only on terminal branches

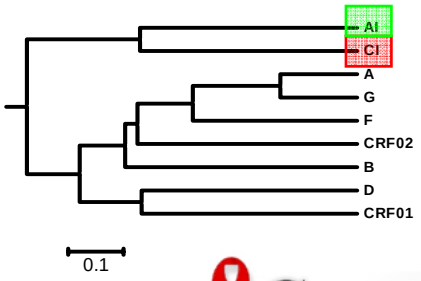
FEL not IFEL

e)



FEL not IFEL

f)



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sites evolving under significantly different selection between AI and CI

- We took all sites where there was any significant evidence of different types of selection happening between AI or CI
- And only sites where these differences were detectable by FEL and not IFEL (where the difference was in the terminal branch)
- We confirmed differences in selection using PARRIS, a random effects likelihood test that assigns a posterior probability of the type of selection at that site

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sites evolving under significantly differential selection between AI and CI

Codon position (HXB2 gp160)	Normalised dN/dS (SLAC, FEL, IFEL)a		PARRIS	Gp41 domain
	AI	CI		
43 (554)	-1.62, **-0.36**, -0.37	0.50, 0.1, 0	no	N-heptad repeat
48 (559)	-3.43, **-0.71**, -0.7	0.43, 0.1, 0	no	N-heptad repeat
105 (616)b	-1.62, **-0.36**, -0.37	0.49, 0.03, 0	YES	Loop region (glyc site)
163 (674)b	-0.07, -0.04, 0.48	**2.88, 0.19, 0.44**	YES	MPER (**4E10** epitope)
300 (804)b	0.22, 0.01, -0.04	**-1.62, -0.16**, -0.18	YES	LLP3
309 (813)b	-0.24, -0.05, -0.04	2.14, **0.19**, 0.24	YES	LLP3

mutations at site 43 are associated with resistance to

[illegible]

the envelope trimer core during fusion

Conclusions

- We show that variations in the selective pressures acting on viruses during the acute and chronic stages of infections are detectable by comparing sequences sampled during these infection phases
- Our analysis reveals clear differences in the distributions of sites evolving under positive and negative selection in chronic and acute subtype C infections
- 4 of 6 the gp41 residues evolving under stronger purifying selection during acute infection are involved in fusion or transmission related functions
- This is suggestive of a selective processes such as a transmission sieve

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Conclusions (cont'd)

- This may be a useful means of identifying viral genetic features that are important for transmission or early infection and thus relevant to rational vaccine or microbicide design

- We aim to further to establish the biological significance of identified sites experimentally

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