CD4 T Cell Decline Is Not Associated With Amino Acid Changes in HIV-1 gp120

Colin Wikholm and Isai Lopez
BIOL 368: Bioinformatics Laboratory
Department of Biology
Loyola Marymount University
November 15, 2016

Outline

- HIV-1 gp120 structure affects infection of host cells, and evasion of host immunity, and virus survival.

- Markham et al. (1998) found that high *gp120* genetic diversity was associated with rapid CD4 T cell decline.

- We analyzed gp120 mutational changes to look for associations between amino acid diversity and rate of CD4 T cell decline.

- We found no significant difference in amino acid diversity between HIV-1 progressor groups.

- Future studies should narrow structural and functional analysis to the V3 region of gp120.

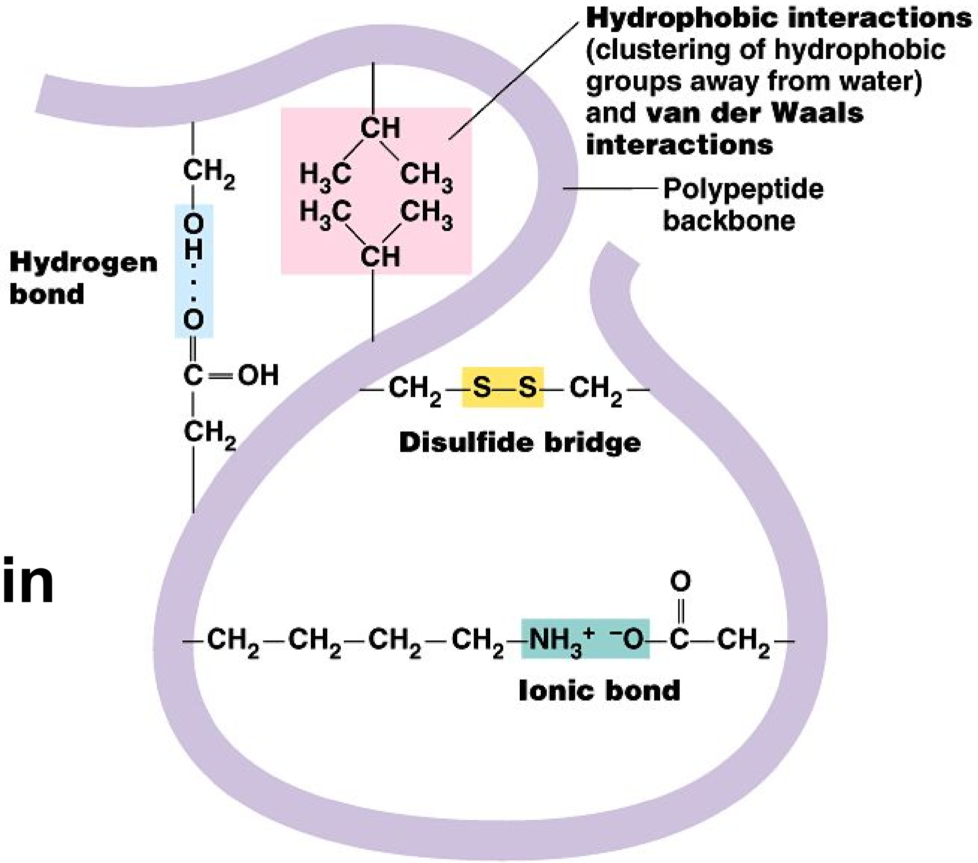
Outline

- **HIV-1 gp120 structure affects infection of host cells, and evasion of host immunity, and virus survival.**
- Markham et al. (1998) found that high *gp120* genetic diversity was associated with rapid CD4 T cell decline.
- We analyzed gp120 mutational changes to look for associations between amino acid diversity and rate of CD4 T cell decline.
- We found no significant difference in amino acid diversity between HIV-1 progressor groups.
- Future studies should narrow structural and functional analysis to the V3 region of gp120.

Protein Primary Structure Determines Protein Folding and Protein Function

- **Nonsynonymous mutations change the the amino acid sequence.**

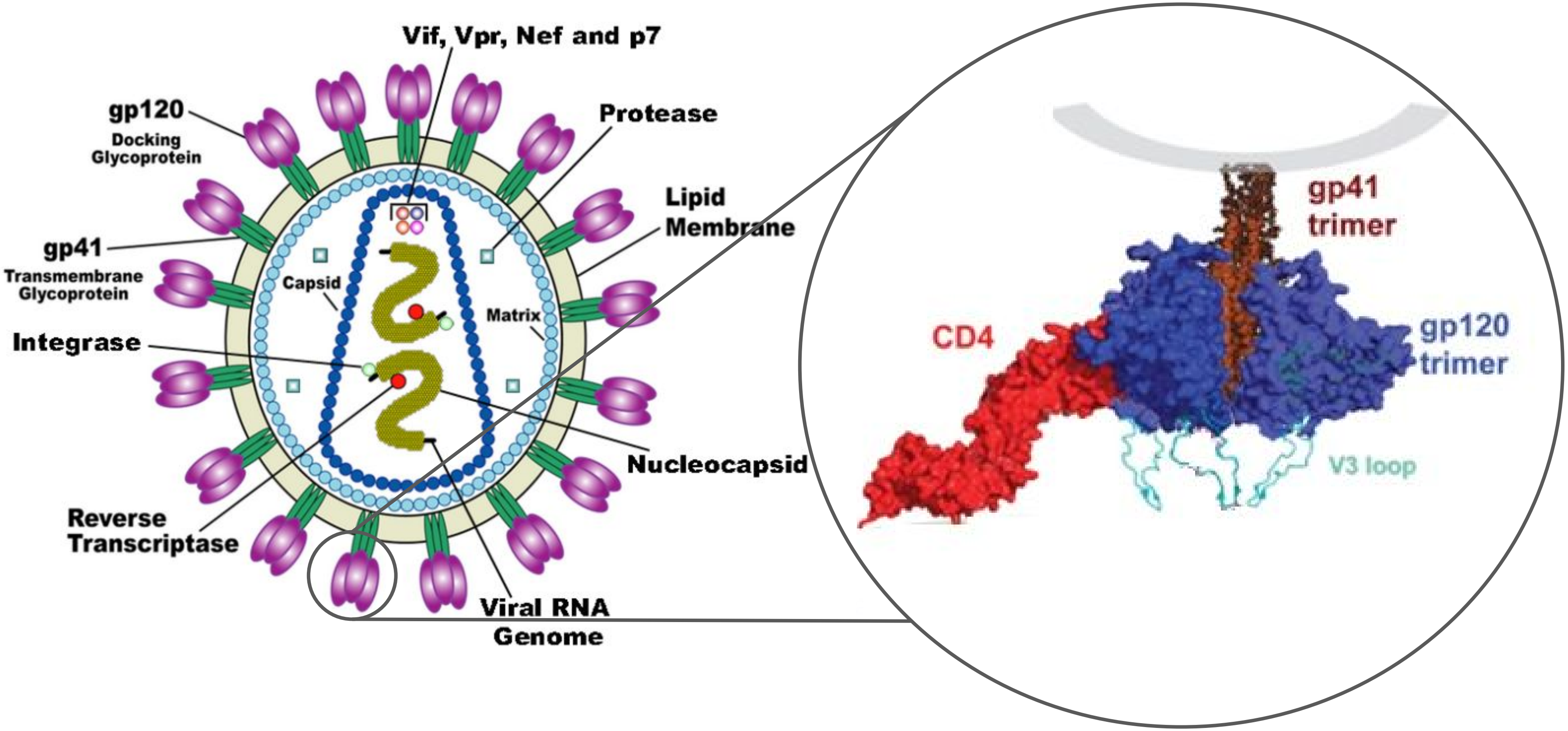
- **Side chains alterations cause changes in protein folding, intermolecular interactions, and structure.**



http://alevelnotes.com/content_images/Image85.gif

HIV-1 Expresses a Series of Surface Proteins for Infection of Host Cells

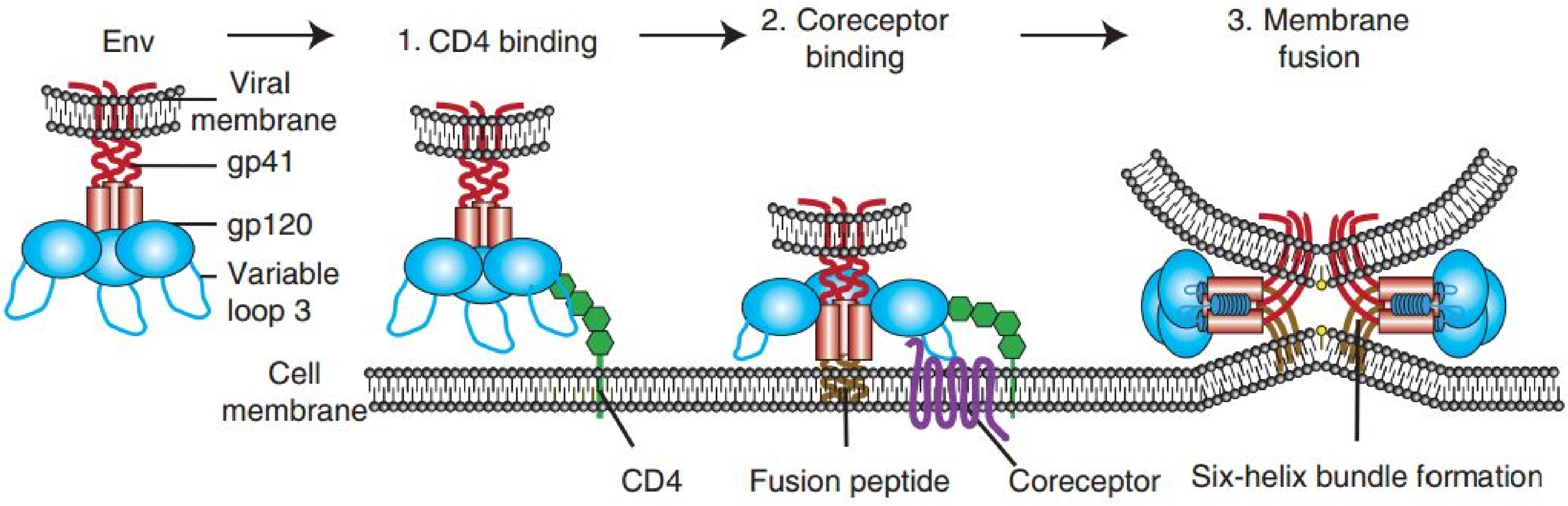
- **The gp160 is cleaved into gp120 and gp41, which together form the viral spike.**



http://people.mpi-inf.mpg.de/~fmueller/thesis/thesis_mueller.pdf

HIV-1 Infection of Host Cell Involves Primary Binding, Secondary Binding, and Membrane Fusion

1. **The gp120 binds to CD4, inducing conformational change in gp120.**
2. **The gp120 binding sites are exposed and CXCR4 binds to gp120.**
3. **The gp41 glycoprotein initiates fusion of virus and cell membranes.**

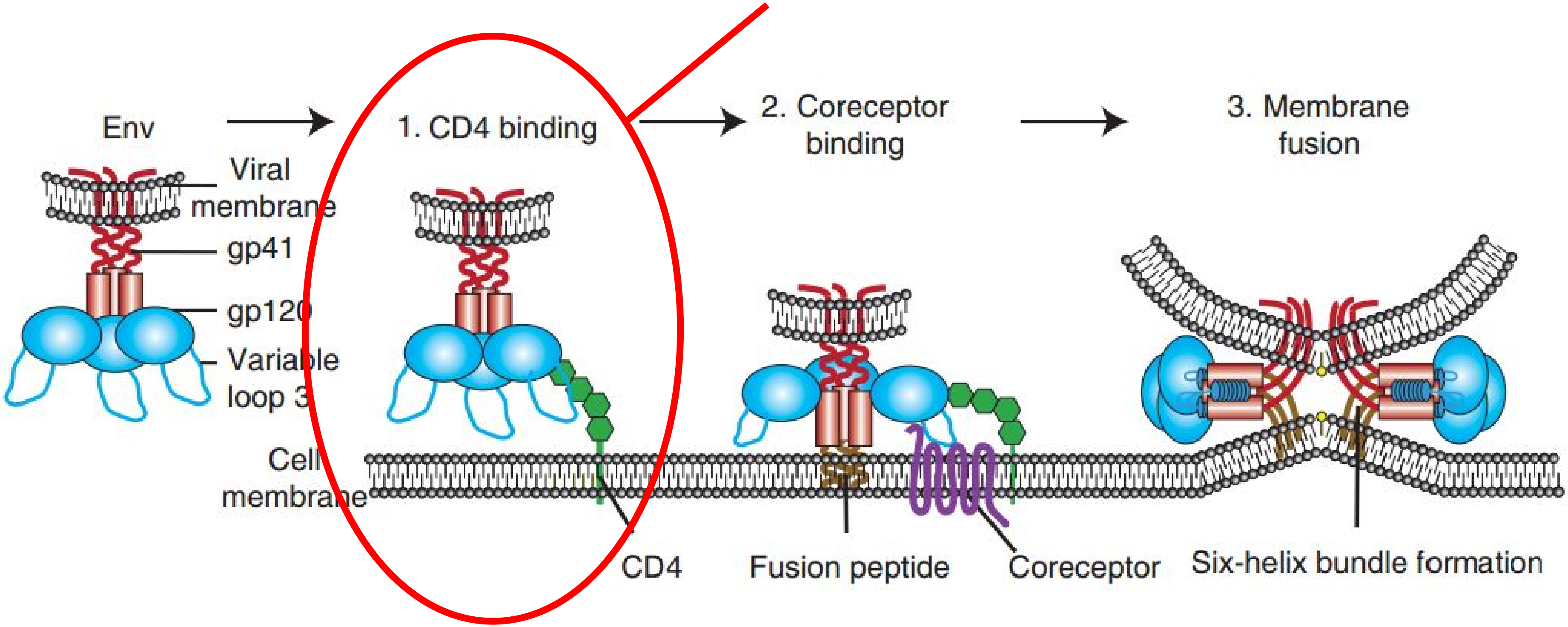


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3405824/

HIV-1 Infection of Host Cell Involves Primary Binding, Secondary Binding, and Membrane Fusion

The gp120 amino acid sequence affects:

a) **Binding to CD4**
b) **Subsequent Chemokine receptor binding**
c) **Neutralization by CD4 induced neutralizing antibodies**



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3405824/

Outline

- HIV-1 gp120 structure affects infection of host cells and evasion of host immunity, and virus survival.

- **Markham et al. (1998) found that high *gp120* genetic diversity was associated with rapid CD4 T cell decline.**

- We analyzed gp120 mutational changes to look for associations between amino acid diversity and rate of CD4 T cell decline.

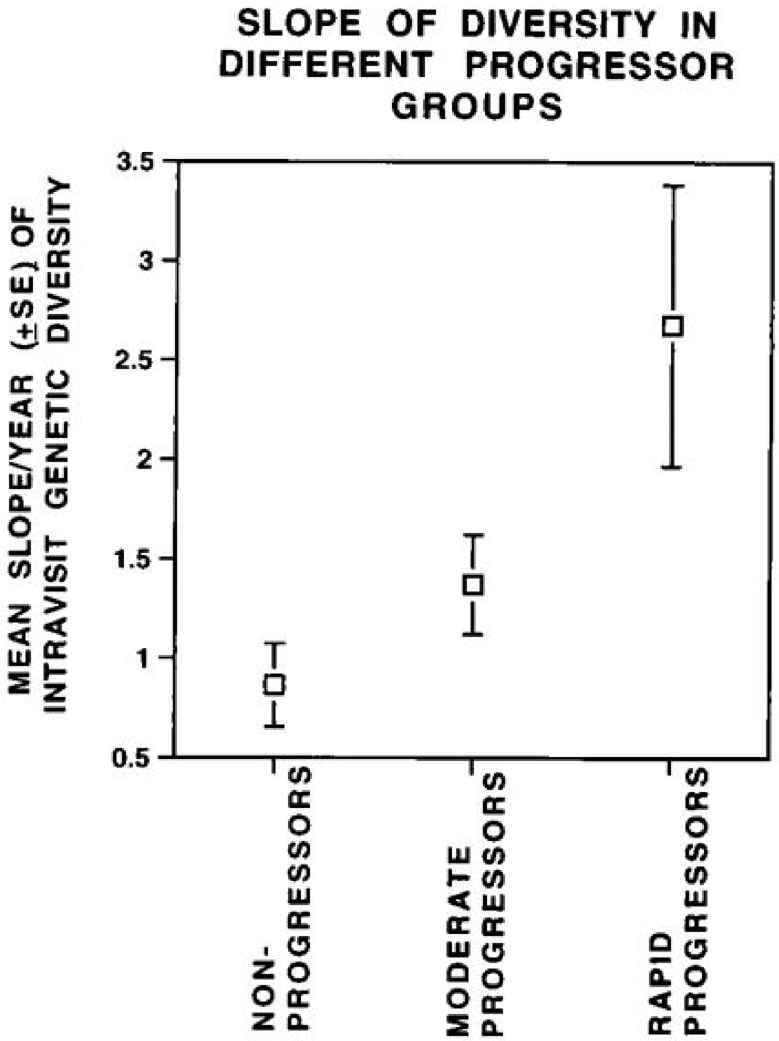
- We found no significant difference in amino acid diversity between HIV-1 progressor groups.

- Future studies should narrow structural and functional analysis to the V3 region of gp120.

Markham et al. (1998) Found Higher *gp120* Diversity in Patients With Rapid CD4 T Cell Decline

- **Would there be higher gp120 amino acid diversity in patients with rapid CD4 T cell decline?**

- **We predicted that patients with higher CD4 T cell decline would have higher amino acid diversity at end of the study.**



Outline

- HIV-1 gp120 structure affects infection of host cells and evasion of host immunity, and virus survival.

- Markham et al. (1998) found that high *gp120* genetic diversity was associated with rapid CD4 T cell decline.

- **We analyzed gp120 mutational changes to look for associations between amino acid diversity and rate of CD4 T cell decline.**

- **We found no significant difference in amino acid diversity between HIV-1 progressor groups.**

- Future studies should narrow structural and functional analysis to the V3 region of gp120.

We Studied Amino Acid Diversity of Two Clones From Each Subject

- **Diversity was calculated in clones within first and last visits, and compared between first and last visits.**

- **Diversity and change in diversity was compared for strong, weak, and no consensus amino acid positions.**
  - Total number of non-fully-conserved regions were also analyzed.

- **Visualization of the gp120 protein structure was used to supplement statistical analysis.**

Differences in Amino Acid Diversity and Change in Amino Acid Diversity Were Not Significant

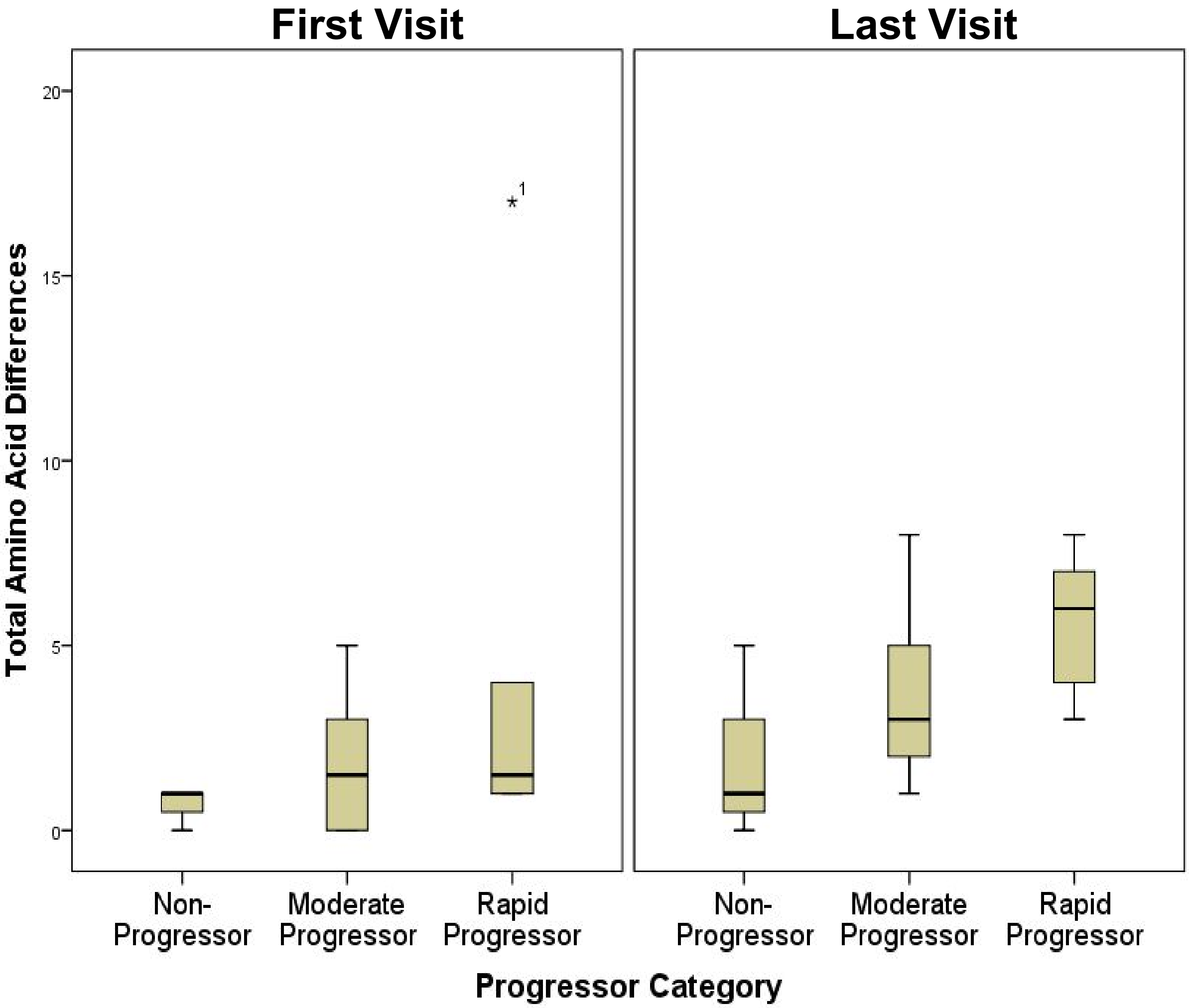
- **Visit and progressor type was not significantly associated with types of mutation regions.**
  - Progressor type still indicates possible relationship.

P-Values of Two-Way Anova Tests

	Strong	**Weak**	**Non-Consensus**	**All Non-Fully-Conserved**
First vs. Last Visit	.284	.574	.413	.266
Progressor Type	.074	.267	.187	.094
Combined Interactions	.692	.109	.633	.981

Differences in Amino Acid Diversity and Change in Amino Acid Diversity Were Not Significant

- **Box plot confirms diversity trend depending on progressor type.**
- **Inter-visit mutational diversity shows little dependence on visit.**



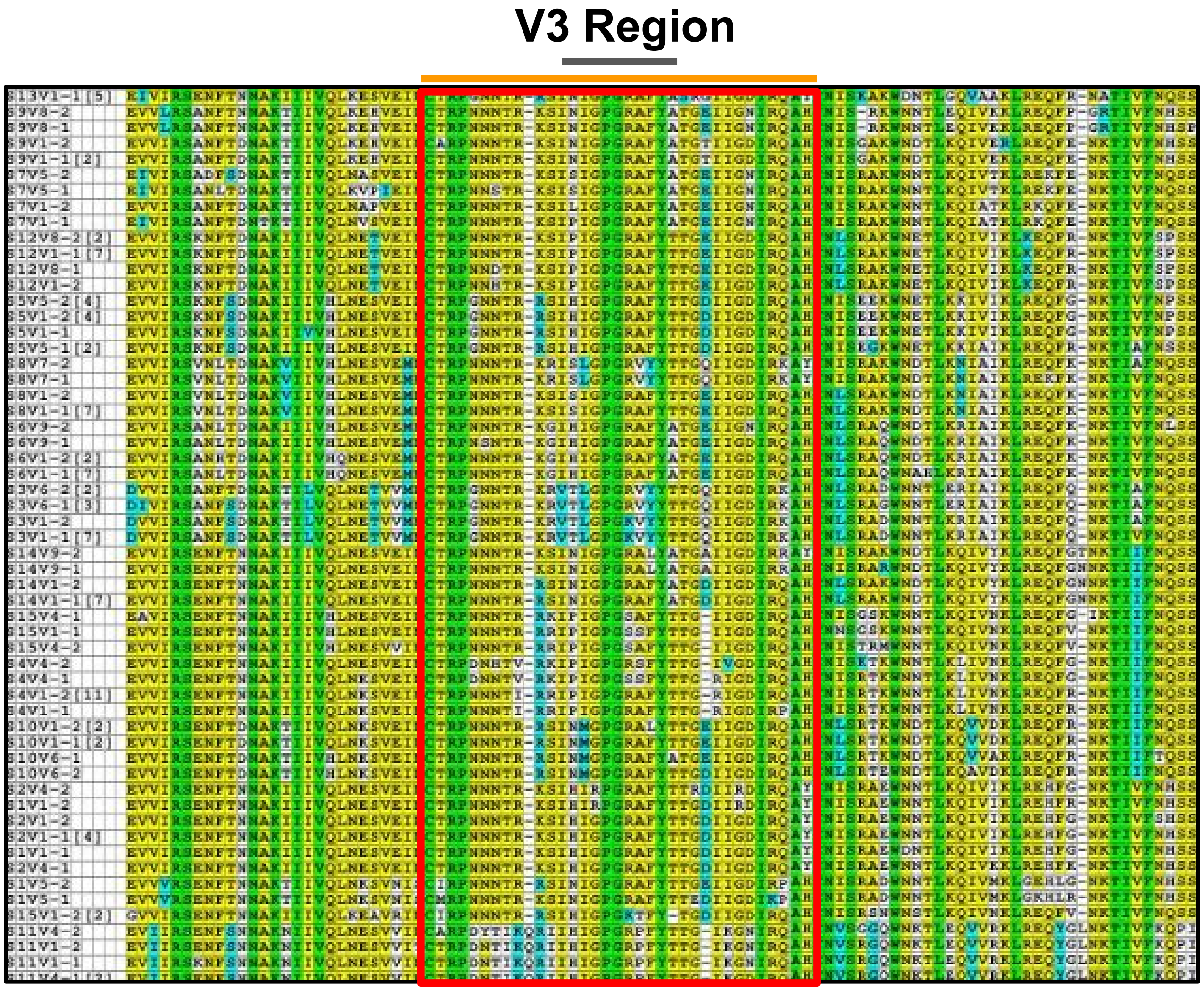
Sequence Alignment Shows Uneven Distribution of Mutational Regions

Identical

Different

Similar

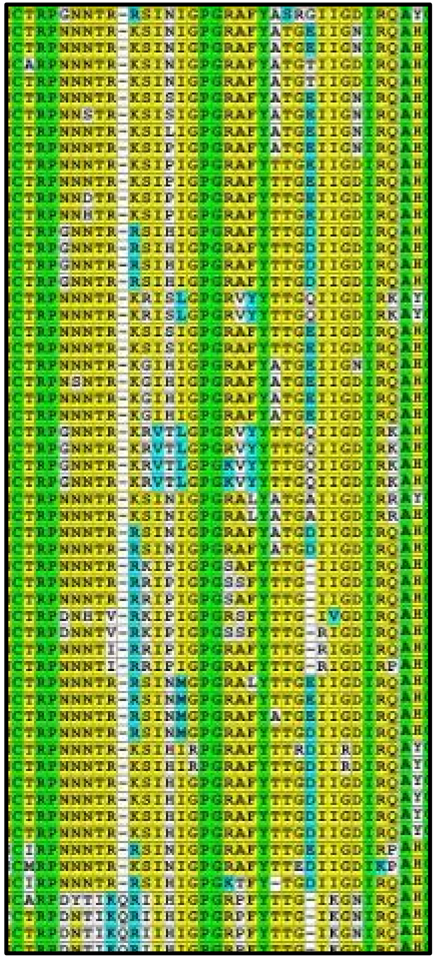
Completely conserved



- The V3 region shows overall low levels of conservation, but one central conserved region

Sequence Alignment Shows Uneven Distribution of Mutational Regions

V3 Region



```
CTRPGNNTR-RSINIGPGRAFYASRGIIGDIRQAY
CTRPNNNTR-KSINIGPGRAFYATGEIIGNIRQAH
CTRPNNNTR-KSINIGPGRAFYATGEIIGNIRQAH
CARPNNNTR-KSINIGPGRAFYATGTIIGDIRQAH
CTRPNNNTR-KSINIGPGRAFYATGTIIGDIRQAH
CTRPNNNTR-KSISIGPGRAFYATGEIIGNIRQAH
CTRPNNSTR-KSISIGPGRAFYATGKIIGNIRQAH
CTRPNNNTR-KSILIGPGRAFYATGEIIGNIRQAH
CTRPNNNTR-KSIPIGPGRAFYATGEIIGNIRQAH
CTRPNNNTR-KSIPIGPGRAFYTTGKIIGDIRQAH
CTRPNNNTR-KSIPIGPGRAFYTTGEIIGDIRQAH
CTRPNNDTR-KSIPIGPGRAFYTTGKIIGDIRQAH
CTRPNNHTR-KSIPIGPGRAFYTTGKIIGDIRQAH
CTRPGNNTR-RSIHIGPGRAFYTTGDIIGDIRQAH
CTRPGNNTR-RSIHIGPGRAFYTTGDIIGDIRQAH
CTRPGNNTR-RSIHIGPGRAFYTTGDIIGDIRQAH
CTRPGNNTR-RSIHIGPGRAFYTTGDIIGDIRQAH
CTRPNNNTR-KRISLGPGRVYYTTGQIIGDIRKAY
CTRPNNNTR-KRISLGPGRVYYTTGQIIGDIRKAY
CTRPNNNTR-KSISIGPGRAFYTTGEIIGDIRQAH
CTRPNNNTR-KSISIGPGRAFYTTGKIIGDIRQAH
CTRPNNNTR-KGIHIGPGRAFYATGEIIGNIRQAH
CTRPNSNTR-KGIHIGPGRAFYATGEIIGDIRQAH
CTRPNNNTR-KGIHIGPGRAFYATGKIIGDIRQAH
CTRPNNNTR-KGIHIGPGRAFYATGEIIGDIRQAH
CTRPGNNTR-KRVTLGPGRVYYTTGQIIGDIRKAH
CTRPGNNTR-KRVTLGPGRVYYTTGQIIGDIRKAH
CTRPGNNTR-KRVTLGPGKVYYTTGQIIGDIRKAH
CTRPGNNTR-KRVTLGPGKVYYTTGQIIGDIRKAH
CTRPNNNTR-KSINIGPGRALYATGAIIGDIRRAY
CTRPNNNTR-KSINIGPGRALYATGAIIGDIRRAH
CTRPNNNTR-RSINIGPGRAFYATGDIIGDIRQAH
CTRPNNNTR-RSINIGPGRAFYATGDIIGDIRQAH
CTRPNNNTR-RKIPIGPGSAFYTTG-IIGDIRQAH
CTRPNNNTR-RRIPIGPGSSFYTTG-IIGDIRQAH
CTRPNNNTR-RRIPIGPGSAFYTTG-IIGDIRQAH
CTRPDNHTV-RKIPIGPGRSFYTTG-IVGDIRQAH
CTRPDNNTV-RKIPIGPGSSFYTTG-RIGDIRQAH
CTRPNNNTI-RRIPIGPGRAFYTTG-RIGDIRQAH
CTRPNNNTI-RRIPIGPGRAFYTTG-RIGDIRPAH
CTRPNNNTR-RSINMGPGRALYTTGEIIGDIRQAH
CTRPNNNTR-RSINMGPGRAFYTTGEIIGDIRQAH
CTRPNNNTR-RSINMGPGRAFYATGEIIGDIRQAH
CTRPNNNTR-RSINMGPGRAFYTTGDIIGDIRQAH
CTRPNNNTR-KSIHIRPGRAFYTTRDIIRDIRQAY
CTRPNNNTR-KSIHIRPGRAFYTTGDIIRDIRQAY
CTRPNNNTR-KSIHIGPGRAFYTTGDIIGDIRQAY
CTRPNNNTR-KSIHIGPGRAFYTTGDIIGDIRQAY
CTRPNNNTR-KSIHIGPGRAFYTTGDIIGDIRQAY
CTRPNNNTR-KSIHIGPGRAFYTTGDIIGDIRQAY
CIRPNNNTR-RSINIGPGRAFYTTGEIIGDIRPAH
CMRPNNNTR-KSINIGPGRAFYTTEDIIGDIKPAH
CIRPNNNTR-RSIHIGPGKTFY-TGDIIGDIRQAH
CARPDYTIKQRIIHIGPGRPFYTTG-IKGNIRQAH
CTRPDNTIKQRIIHIGPGRPFYTTG-IKGNIRQAH
CTRPDNTIKQRIIHIGPGRPFYTTG-IKGNIRQAH
```

Identical

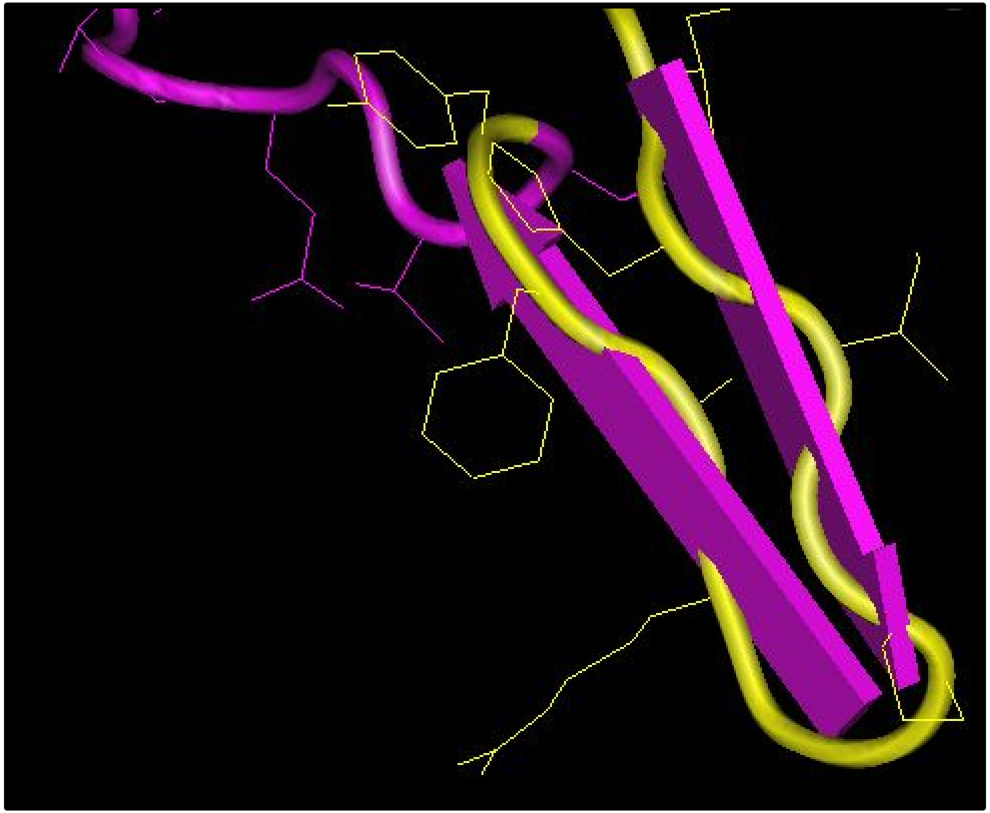
Different

Similar

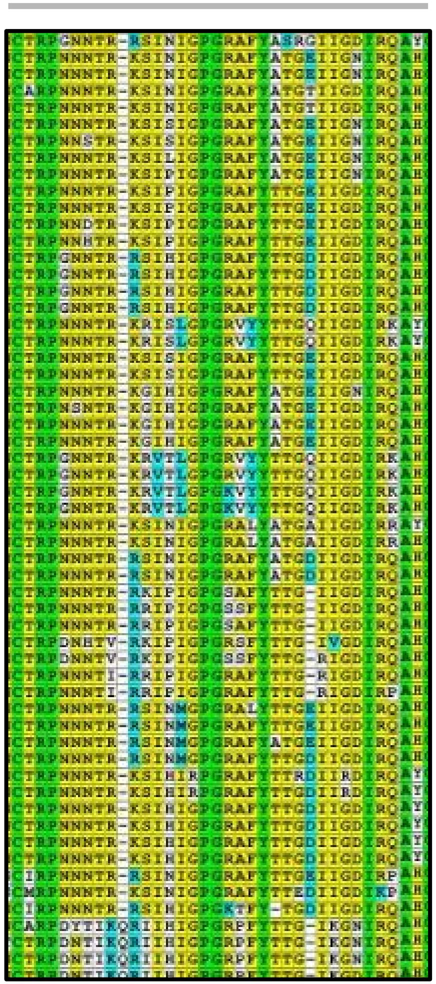
Completely conserved

Sequence Alignment and Structure Analysis Confirm Variable Mutation Distribution Within the V3 Region

- **Previous studies show the most variability in the dual β-sheets of the V3 region (Zolla-Pazner & Cardozo 2010)**



V3 Region



Identical

Different

Similar

Completely conserved

Outline

- HIV-1 gp120 structure affects infection of host cells and evasion of host immunity, and virus survival.
- Markham et al. (1998) found that high *gp120* genetic diversity was associated with rapid CD4 T cell decline.
- We analyzed gp120 mutational changes to look for associations between amino acid diversity and rate of CD4 T cell decline.
- We found no significant difference in amino acid diversity between HIV-1 progressor groups.
- **Future studies should narrow structural and functional analysis to the V3 region of gp120.**

Limitations of this Analysis Suggest Future Studies Should Focus on Larger Sample Sizes and the V3 region

- **Subject 15 seems to be possible outlier in first and final visit**
  - Markham at al. (1998) noted subject 15 as having unusually high genetic diversity.

- **The sample size for this investigation limited each subject to two clones per visit.**
  - Continued investigation should test each clone.

- **This study tested the entire gp120 region sequenced by Markham at al. (1998).**
  - Structural analysis shows uneven mutation distribution.
  - Future structural analysis of HIV-1 should focus on the amino acid sequence composing the dual β-strands of the V3 region.

Acknowledgments

Dr. Kam D. Dahlquist
LMU Department of Biology

References

Zolla-Pazner, S., & Cardozo, T. (2010). STRUCTURE–FUNCTION RELATIONSHIPS OF HIV-1 ENVELOPE SEQUENCE-VARIABLE REGIONS PROVIDE A PARADIGM FOR VACCINE DESIGN. *Nature Reviews. Immunology*, *10*(7), 527–535. http://doi.org/10.1038/nri2801