

Analysis of HIV-1 Amino Acid Sequences Suggests No Correlation Between Diversity and Progressor Groups

**Mia Huddleston and Avery Vernon-Moore
BIOL 368: Bioinformatics Laboratory
Loyola Marymount University
November 15, 2016**

Outline

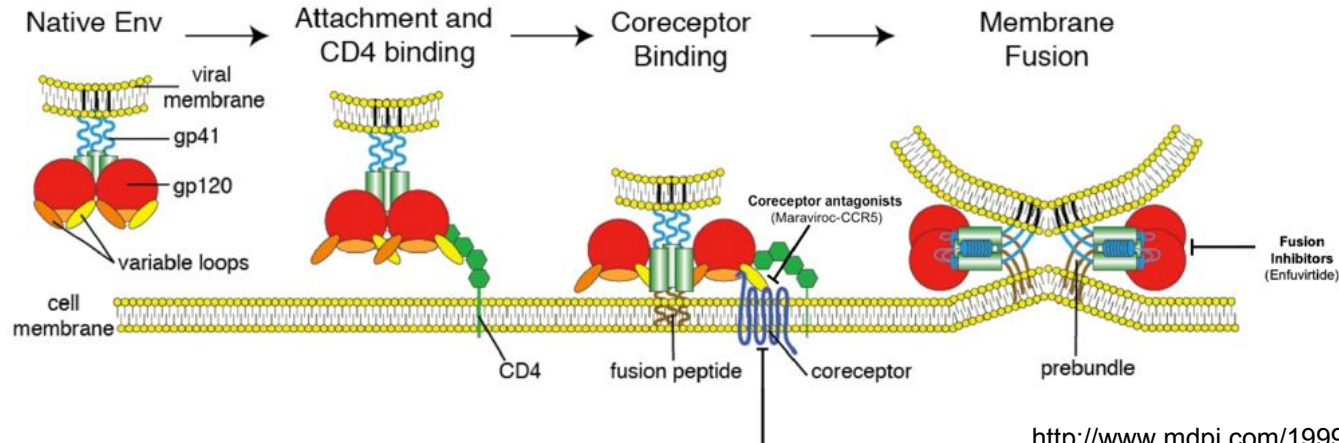
- **The HIV virus attacks CD4 T cells in the human body and can mutate its genetic structure through changes in amino acids**
- **Markham et al. 1998 concluded that there was higher diversity among rapid progressor groups**
- **Amino acid sequences of the first and last visits of four subjects from Markham et al. 1998 were analyzed**
- **No significant difference was observed between number of mutations observed in rapid versus nonprogressors**
- **Certain types of mutations may affect the structure of gp120 more dramatically than others**
- **Future studies consisting of subjects with larger numbers of data points are needed to determine higher rates of diversity among rapid progressors**

Outline

- **The HIV virus attacks CD4 T cells in the human body and can mutate its genetic structure through changes in amino acids**
- Markham et al. 1998 concluded that there was higher diversity among rapid progressor groups
- Amino acid sequences of the first and last visits of four subjects from Markham et al. 1998 were analyzed
- No significant difference was observed between number of mutations observed in rapid versus non-progressors
- Certain types of mutations may affect the structure of gp120 more dramatically than others
- Future studies consisting of subjects with larger numbers of data points are needed to determine higher rates of diversity among rapid progressors

When the HIV virus enters a human host it attacks and infects CD4 T cells.

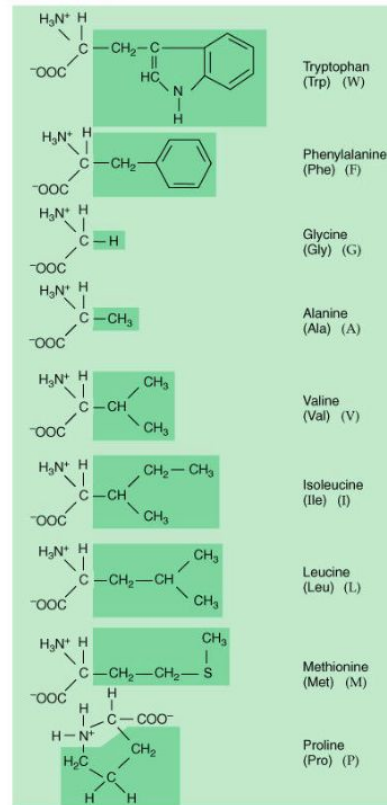
- HIV viruses infect CD4 T cells in human hosts enabling them to proliferate
 - A CD4 T cells' role is to protect the body from infection
 - When a CD4 T cell is infected with an HIV virus, it cannot fight infections
- The V3 loop on the gp120 of a HIV-1 virus helps to infect the host cell by binding to a cytokine receptor



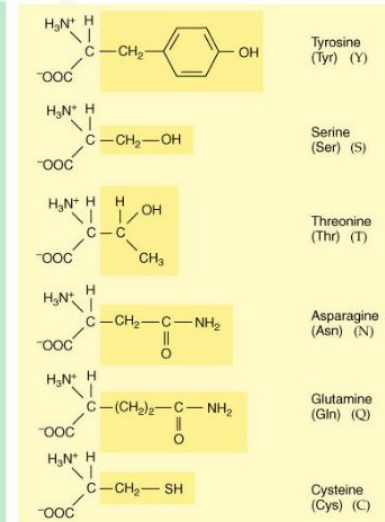
Amino acid mutations can change the structure of the HIV virus.

- HIV viruses actively mutate their genetic structure in the body making it difficult to create drugs to kill the virus
- Amino acids are most often categorized into four sections
 - Nonpolar, Polar, Basic, and Acidic

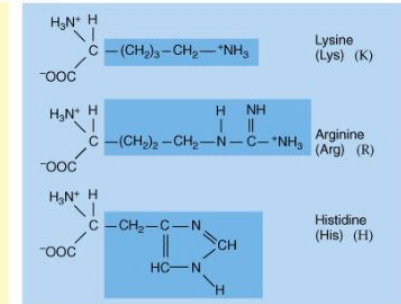
Neutral, nonpolar



Neutral, polar



Basic



Acidic



Outline

- The HIV virus attacks CD4 T cells in the human body and can mutate its genetic structure through changes in amino acids
- **Markham et al. 1998 concluded that there was higher diversity among rapid progressor groups based on nucleotide sequences**
- Amino acid sequences of the first and last visits of four subjects from Markham et al. 1998 were analyzed
- No significant difference was observed between number of mutations observed in rapid versus nonprogressors
- Certain types of mutations may affect the structure of gp120 more dramatically than others
- Future studies consisting of subjects with larger numbers of data points are needed to determine higher rates of diversity among rapid progressors

Markham et al. 1998 showed results concluding higher diversity was found among rapid progressors compared to nonprogressors.

- **15 subjects were compared and placed into categories of nonprogressors, moderate progressors, and rapid progressors based on CD4 T cell decline**
- **Diversity between the clones of each subject was determined by comparing the number of nucleotide sequence mutations**
- **Rapid progressors were determined to have a higher diversity**

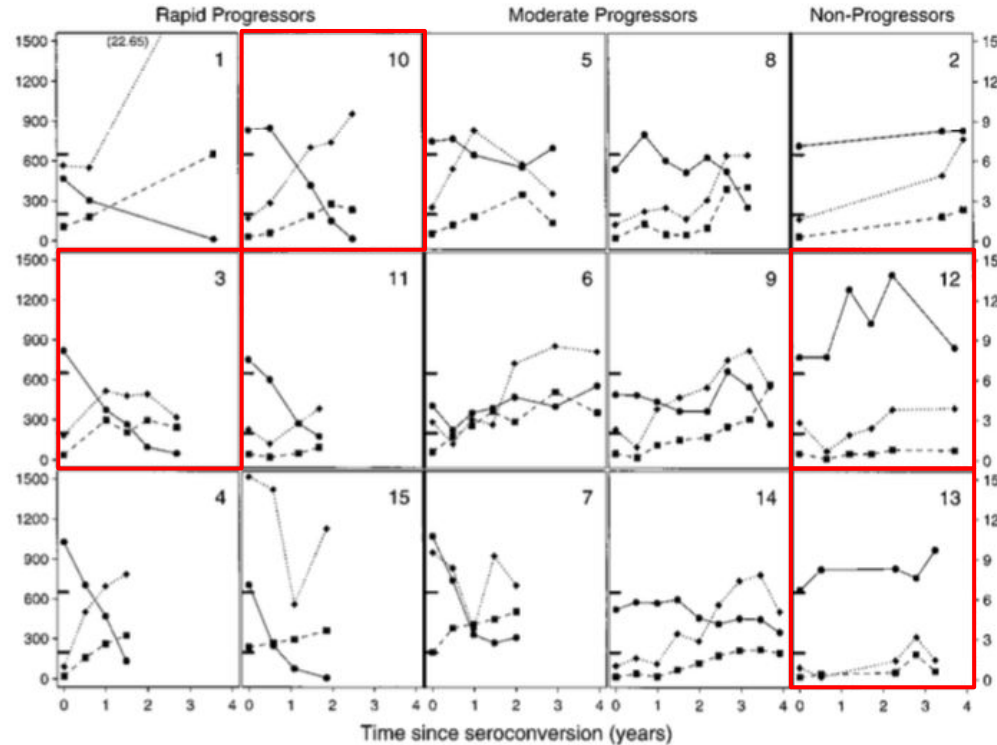
Does diversity increase within the amino acid sequences over time more within the rapid progressors compared to nonprogressors?

- **Hypothesis: Since diversity increases over time within the DNA sequences for all progressor types, and more so within the rapid progressors, we would expect to see more diversity within the amino acid sequences over time within the rapid progressors.**
 - **The amino acid sequences in the first and last visits for four subject were analyzed**
 - **Using the multiple sequence alignment and Clustaldist tools within Biology Workbench the first and last sequences were compared**
 - **Amino acid changes were analyzed based on severity**
 - **The locations of the mutations were observed using a structure of gp120 created by Huang et al.**

Outline

- The HIV virus attacks CD4 T cells in the human body and can mutate its genetic structure through changes in amino acids
- Markham et al. 1998 concluded that there was higher diversity among rapid progressor groups
- **Amino acid sequences of the first and last visits of four subjects from Markham et al. 1998 were analyzed**
- No significant difference was observed between number of mutations observed in rapid versus nonprogressors
- Certain types of mutations may affect the structure of gp120 more dramatically than others
- Future studies consisting of subjects with larger numbers of data points are needed to determine higher rates of diversity among rapid progressors

Two subjects from the nonprogressor and rapid progressor groups were chosen comparing each of their first and last visit clones.



The overall number of mutations per subject were calculated using multiple sequence alignment.

Group	Subject	Number of Clones	Number of Mutations
Nonprogressors	Subject 13	10	5
Nonprogressors	Subject 12	12	11
Rapid Progressors	Subject 3	10	9
Rapid Progressors	Subject 10	17	24

Sequence alignment of rapid progressors shows large number of mutations from first to last

```
S3V6-6  DIVIRSANFSDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G R V Y Y T T
S3V6-3  DIVIRSANFSDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G R V Y Y T T
S3V6-1[3] DIVIRSANFSDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G R V Y Y T T
S3V6-5[2] DIVIRSANFSDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G R V Y Y T T
S3V6-4  DIVIRSANFSDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G R V Y Y T T
S3V6-2[2] DVVIRSANFTDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G R V Y Y T T
S3V1-4  DVVIRSANFTDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G K V Y Y T T
S3V1-3  DVVIRSANFTDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G K V Y Y T T
S3V1-1[7] DVVIRSANFSDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G K V Y Y T T
S3V1-2  DVVIRSANFSDNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G K V Y Y T T
consensus DiVIRSANFsdNAKTILVQLNETVVMNCTRPGNNT RKRVT L G P G R V Y Y T T

S3V6-6  GQIIGDIRKAHCNLSRAGWNSTLERIAIKLREQFQNKTI AFNQSS
S3V6-3  GQIIGDIRKAHCNLSRAGWNTTLERIAIKLREQFQNKTI AFNQSS
S3V6-1[3] GQIIGDIRKAHCNLSRAGWNTTLERIAIKLREQFQNKTI AFNQSS
S3V6-5[2] GQIIGDIRKAHCNLSRADWNNTLERIAIKLREQFQNKTI AFNQSS
S3V6-4  GQIIGDIRKAHCNLSRADWNNTLERIAIKL-REQFQNKTI GFNQSS
S3V6-2[2] GQIIGDIRKAHCNLSRADWNNTLERIAIKLREQFQNKTI AFNQSS
S3V1-4  GQIIGDIRKAHCNLSRADWNNTLKRIAIKLREQFQNKTI VFNQSS
S3V1-3  GQIIGDIRKAHCNLSRADWNNTLKRIAIKLREQFQNKTI AFNQSS
S3V1-1[7] GQIIGDIRKAHCNLSRADWNNTLKRIAIKLREQFQNKTI VFNQSS
S3V1-2  GQIIGDIRKAHCNLSRADWNNTLKRIAIKLREQFQNKTI AFNQSS
consensus GQIIGDIRKAHCNLSRADWNnTLERIAIKLrEQFQNKTIaFNQSS
```

Sequence alignment of rapid progressors shows large number of mutations from first to last

```

S10V6-9  EVVIRSENFPTDNARTIIIVHLNRSVEINCTRPNNNTRRSINMGPGRVFYATGEIIGDIRQA
S10V6-1  EVVIRSENFPTDNARTIIIVHLNRSVEINCTRPNNNTRRSINMGPGRAFYYATGEIIGDIRQA
S10V6-7  EVVIRSENFPTDNARTIIIVHLNRAVEINCTRPNNNTRRSINMGPGRAFYYATGDIIGDIRQA
S10V6-10 EVVIRSENFPTDNARTIIIVHLNRSVEINCTRPNNNTRRSINMGPGRAFYYATGEIIGDIRQA
S10V6-6  EVAIRSENFPTDNARTIIIVHLNRSVEINCTRPNNNTRRSINMGPGRAFYYATGDIIGDIRQA
S10V6-3  EVVIRSENFPTDNARTIIIVQLNRSVEINCTRPNNNTRRSINMGPGRALYTTGDIIGDIRQA
S10V6-2  EVVIRSENFPTDNARTIIIVHLNRSVEINCTRPNNNTRRSINMGPGRAFYYTTGDIIGDIRQA
S10V1-4  EVVIRSENFPTDNARTIIIVQLNRSVEINCTRPNNNTRRSINMGPGRAFYYTTGEIIGDIRQA
S10V1-3 [2] EVVIRSENFPTDNARTIIIVQLNRSVEINCTRPNNNTRRSINMGPGRAFYYTTGEITGDIRQA
S10V1-7  EVVIRSENFPTDNARTIIIVQLNRSVEINCTRPNNNTRRSINMGPGRAFYYTTGEIIGDIRQA
S10V1-5  EVVIRSENFPTDNARTIIIVQLNRSVEINCTRPNNNTRRSINMGPGRAFYYTTGEIIGDIRQA
S10V1-1 [2] EVVIRSENFPTDNARTIIIVQLNRSVEINCTRPNNNTRRSINMGPGRAFYYTTGEIIGDIRQA
S10V1-6  EVVIRSENFPTDNARTIIIVQLNRSVEINCTRPNNNTRRSINMGPGRAFYYTTGGIIGDIRQA
S10V1-2 [2] EVVIRSENFPTDNARTIIIVQLNRSVEINCTRPNNNTRRSINMGPGRALYTTGEIIGDIRQA
S10V6-8  EVVIRSENFPTDNARTIIIVHLNRSVEINCTRPNNNTRRSINMGPGRVFYTTGEIIGDIRQA
S10V6-5  EVVIRSENFPTDNARTIIIVHLNRSVEINCTRPNNNRKRIRITMGPGRALYTTGEIIGDIRQR
S10V6-4  EVVIRSENFPTDNARTIIIVHLNRSVEINCTRPNNNRKRIRINMGPGRVLYTTGEIIGDIRQR
consensus EVVIRSENFPTDNARTIIIVhLNk#VEINcTRPNNNTRr#InMGPGRaFYtTGeIIGDIRQa

```

```

S10V6-9  HCNLSRTKWNNTLQVVARLREQFRNRTIIFTQSS
S10V6-1  HCNLSRTKWNNTLQVVARLREQFRNRTIIFTQSS
S10V6-7  HCNLSRTKWNNTLQAVARLREQFRNRTIIFTQSS
S10V6-10 HCNLSRTKWNNTLQVVDRLREQFRNRTIIFTQSS
S10V6-6  HCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V6-3  HCNI$RTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V6-2  HCNLSRTKWNNTLQAVDRLREQFRNRTIIFNQSS
S10V1-4  HCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V1-3 [2] HCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V1-7  HCNLSRTKWNNTLQVVDRLGEQFRNRTIIFNQSS
S10V1-5  HCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V1-1 [2] HCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V1-6  HCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V1-2 [2] HCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V6-8  HCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
S10V6-5  YCNLSRTKWNNTLQGADRLREQFRNRTIIFNQSS
S10V6-4  YCNLSRTKWNNTLQVVDRLREQFRNRTIIFNQSS
consensus hCNLSRTkWNNTLQvvdRLrEQFRNRTIIFnQSS

```

Sequence alignment of nonprogressor 12 shows a slightly lower number of mutations per number of clones than rapid progressors collectively.

```
S12V8-1 EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V1-2 EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V8-8 EVVIRSRNFADNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V1-3 EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V1-1 [7] EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V8-2 [2] EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V8-3 [2] EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V8-4 EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V1-4 EVVIRSVNFTDNART IIVQLNTSVEINCTRPNNDTRRSIP IGPGRAFYTTGEIIGDIRQA
S12V8-7 EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYATGEIIGDIRQA
S12V8-6 EVVIRSRNFTDNARI IIVQLNETVEINCTRPNNDTRRSIP IGPGRAFYATGEIIGDIRQA
S12V8-5 EVVIRSVNFTDNART IIVQLNETVEINCTRPNNDTRRSIHIGPGRAFYATGEIIGDIRQA
consensus EVVIRSkNFtDNARI IIVQLNetVEINCTRPNNDTRRSIp IGPGRAFYtTGEIIGDIRQA
```

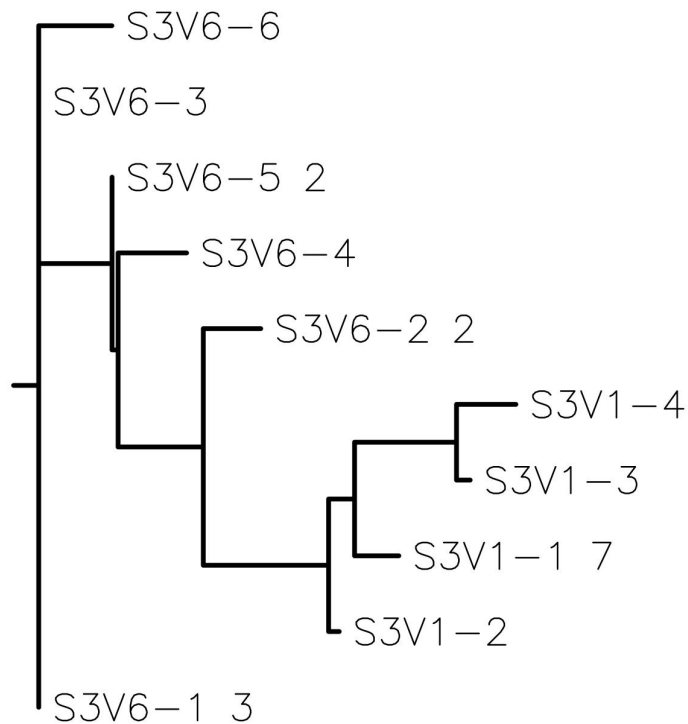
```
S12V8-1 BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V1-2 BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V8-8 BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V1-3 BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPFS
S12V1-1 [7] BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V8-2 [2] BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V8-3 [2] BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V8-4 BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V1-4 BCNLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V8-7 BCTLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
S12V8-6 BCTLSRARWDETLRQIVIKLREQPRNKTIVFSPSS
S12V8-5 BCTLSRARWNETLRQIVIKLREQPRNKTIVFSPSS
consensus BCnLSRARWnETLRQIVIKLREQPRNKTIVFSPsS
```


Sequence alignment of nonprogressor 13 shows less mutations than all other subjects observed.

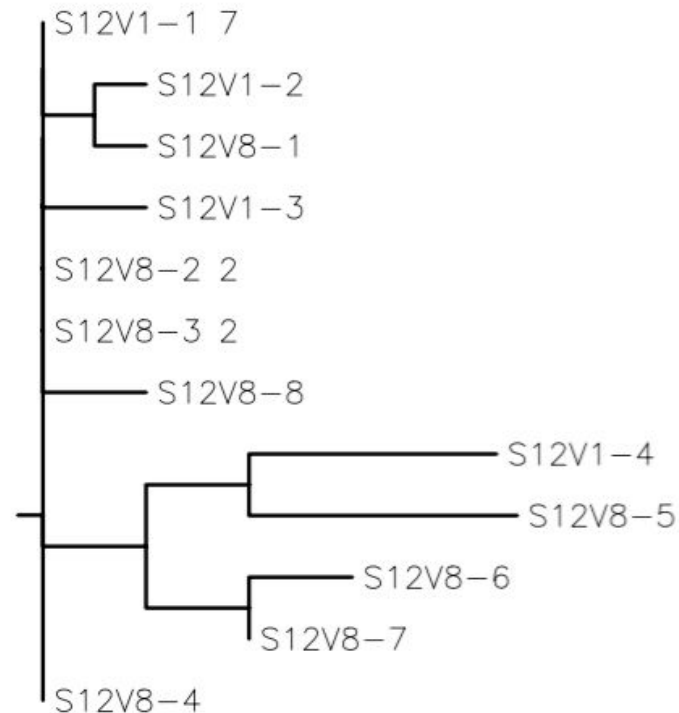
```
S13V1-1[5] EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
S13V1-2[3] EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
S13V1-3 EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
S13V1-4 EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIMGPGRAPYASRGIIGDIRQA
S13V5-1[4] EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
S13V5-2 EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
S13V5-3 EIVIRFENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
S13V5-4[2] EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
S13V5-5 EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
S13V5-6 EIVIRSENFTNNARTI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA
consensus EIVIRSENFTNNARI IIVQLKESVEINCTRPGNNTRRSINIGPGRAPYASRGIIGDIRQA

S13V1-1[5] YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V1-2[3] YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V1-3 YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V1-4 YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V5-1[4] YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V5-2 YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V5-3 YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V5-4[2] YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V5-5 YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
S13V5-6 YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
consensus YCNISRAKWDNTLGQVAARKLREQFRNATIVFNQSS
```

Rooted trees demonstrated no discernable difference between rapid and nonprogressors.



Rapid progressor 3



Nonprogressor 12

Outline

- The HIV virus attacks CD4 T cells in the human body and can mutate its genetic structure through changes in amino acids
- Markham et al. 1998 concluded that there was higher diversity among rapid progressor groups
- Amino acid sequences of the first and last visits of four subjects from Markham et al. 1998 were analyzed
- **No significant difference was observed between number of mutations observed in rapid versus nonprogressors**
- Certain types of mutations may affect the structure of gp120 more dramatically than others
- Future studies consisting of subjects with larger numbers of data points are needed to determine higher rates of diversity among rapid progressors

Clustaldist shows higher rate of diversity among rapid progressors by comparing the distances of mutations from the first visit to the last per subject.

Progressor Type	Subject	Distance between first and last visit
Rapid	3	0.010
Rapid	10	0.137
Non	12	0.010
Non	13	0.021

Outline

- The HIV virus attacks CD4 T cells in the human body and can mutate its genetic structure through changes in amino acids
- Markham et al. 1998 concluded that there was higher diversity among rapid progressor groups
- Amino acid sequences of the first and last visits of four subjects from Markham et al. 1998 were analyzed
- No significant difference was observed between number of mutations observed in rapid versus nonprogressors
- **Certain types of mutations may affect the structure of gp120 more dramatically than others**
- Future studies consisting of subjects with larger numbers of data points are needed to determine higher rates of diversity among rapid progressors

Mutations are identified and located using the structure of gp120 discovered by Huang et al.

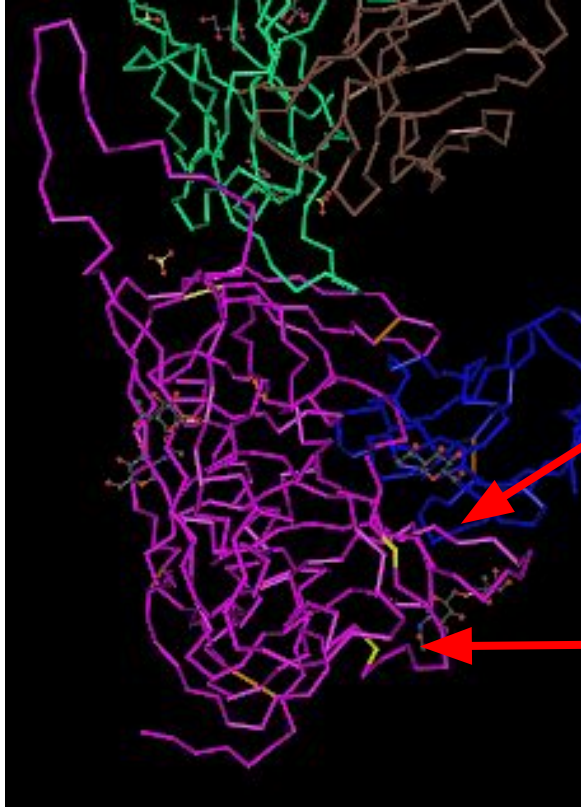


Highlighted in yellow is the V3 region of gp120, the V3 loop can be seen protruding out to the right.

The types of mutations may affect how the protein function would differ.

Progressor type	Subject	Number of noconsensus mutations	Number of clones	Average number of no consensus mutations per clone
Rapid	3	2	10	0.20
Rapid	10	8	17	0.47
Non	12	5	12	0.42
Non	13	3	10	0.30

Although rapid progressor 3 shows less no consensus mutations than expected, a deletion could alter the entire V3 section.



Valine, a nonpolar amino acid mutates to Alanine, also nonpolar, but then mutates to Glycine, a polar amino acid (nonsynonymous mutation)

Arginine is deleted in one of the last visit's clones

Rapid progressor 10 shows multiple mutations on V3 loop that may affect binding.

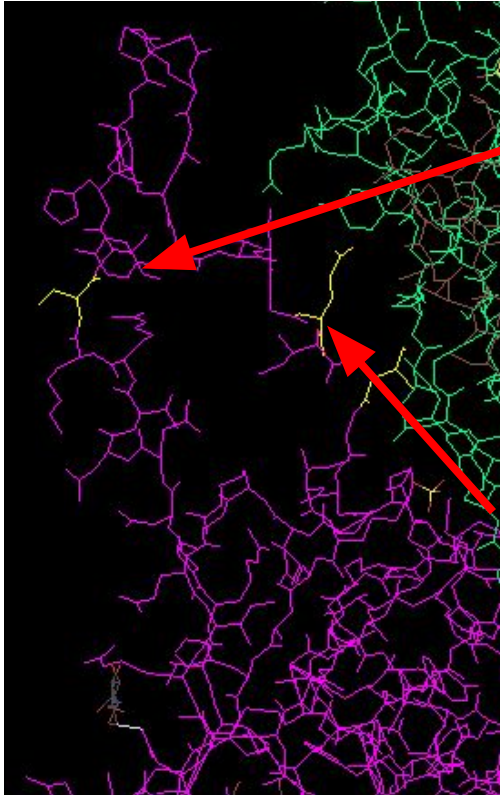
Serine, a polar uncharged amino acid mutates to Arginine, a basic amino acid

Glutamic Acid, an acidic aa mutates to Aspartic Acid, another acidic aa and also mutates to Glycine, a polar uncharged aa

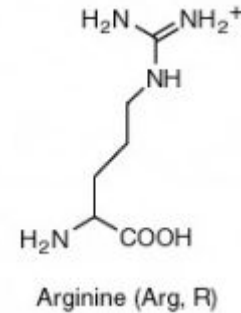
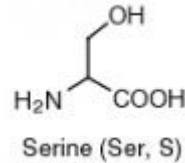
Isoleucine, a nonpolar aa mutates to Threonine a polar uncharged aa (nonsynonymous mutation)



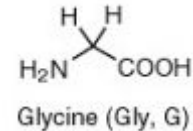
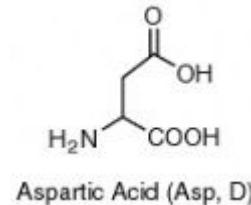
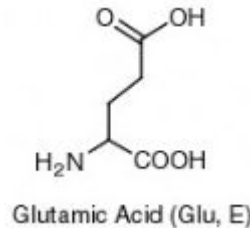
Changes in the structure of each amino acid mutation can affect the severity of the mutation in rapid progressor 10.



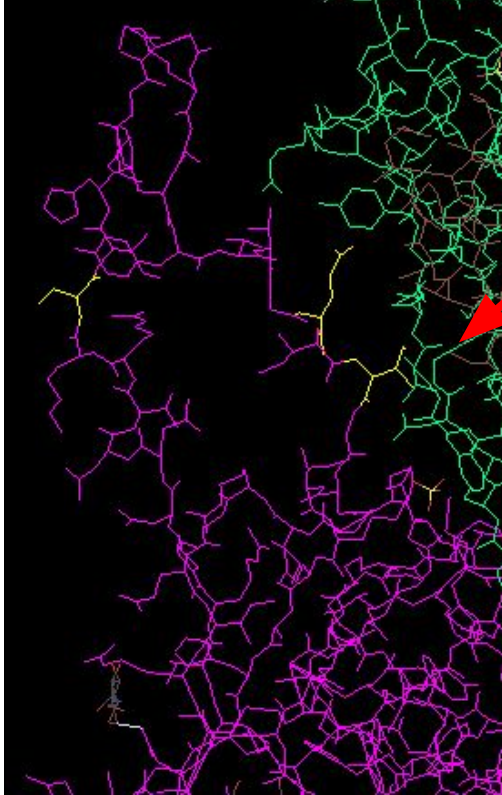
Serine (polar uncharged) to Arginine (basic)



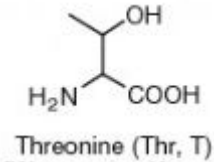
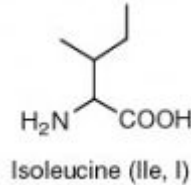
Glutamic Acid (acidic) to Aspartic Acid (acidic) to Glycine (polar)



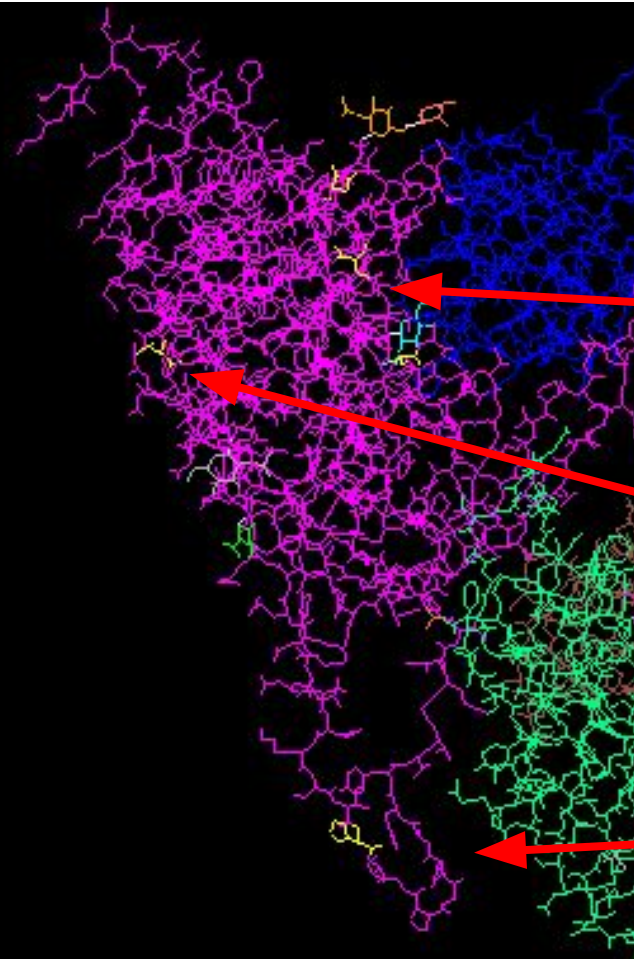
Changes in the structure of each amino acid mutation can affect the severity of the mutation in rapid progressor 10.



Isoleucine (nonpolar) to Threonine (polar uncharged)



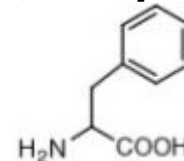
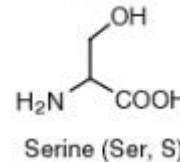
Nonprogressor 12 had a higher number of mutations within the V3 region than nonprogressor 13.



Valine (hydrophobic; nonpolar) to
Lysine (positively charged; basic)

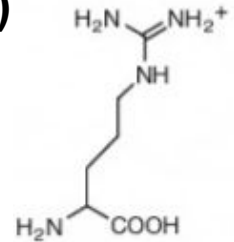
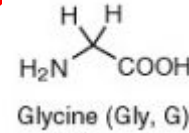
Glutamic Acid (acidic; negatively
charged) to Threonine (polar)

Serine (polar) to Phenylalanine
(hydrophobic; nonpolar)

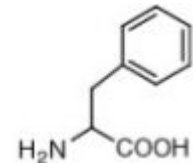
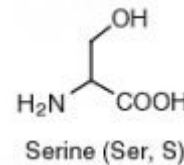


Nonprogressor 13 had fewer mutations which occurred further away from the V3 loop than other subjects.

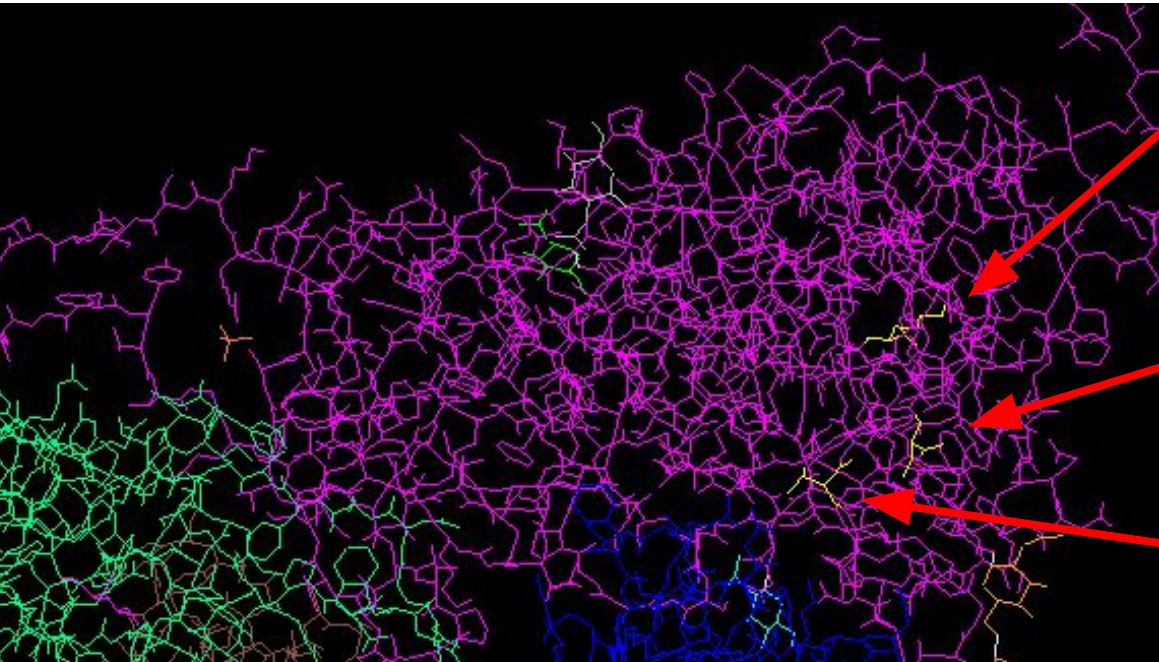
Glycine (hydrophobic) to Arginine (Basic)



Serine (polar) to Phenylalanine (aromatic; hydrophobic)



Isoleucine (hydrophobic) to Methionine (polar)



Outline

- The HIV virus attacks CD4 T cells in the human body and can mutate its genetic structure through changes in amino acids
- Markham et al. 1998 concluded that there was higher diversity among rapid progressor groups
- Amino acid sequences of the first and last visits of four subjects from Markham et al. 1998 were analyzed
- No significant difference was observed between number of mutations observed in rapid versus nonprogressors
- Certain types of mutations may affect the structure of gp120 more dramatically than others
- **Future studies consisting of subjects with larger numbers of data points are needed to determine higher rates of diversity among rapid progressors**

Data suggests higher diversity among rapid progressor amino acid sequences than nonprogressors, but a larger sample size is needed to confirm results.

- **No mutation patterns were observed within progressor groups**
- **More mutations occurred within the V3 loop in rapid progressor 10 than the other subjects**
- **Using more subjects would create a larger sample size**
 - **Moderate progressor group was overlooked**
- **More data points per subject could increase statistical evidence**
 - **Subjects from a more consistent study could be used**

Summary

- **Mutations which occur within the amino acid sequences of HIV-1 can affect the structure and overall function of the virus**
- **Markham et al. 1998 concluded that there was higher diversity among rapid progressor groups**
- **Mutations within amino acid sequences of progressors and nonprogressors from Markham et al. 1998 were analyzed**
 - **Different types of mutations may affect the structure of gp120 more drastically than others**
- **No significant difference was observed between number of mutations observed in rapid versus nonprogressors**
- **To gather more conclusive data, subjects with larger numbers of data points would be needed to determine if there is a higher rate of diversity among the rapid progressor group**

Acknowledgements

We would like to thank Dr. Dahlquist and the Loyola Marymount Department of Biology for their help.

References

Huang, C. C., Tang, M., Zhang, M. Y., Majeed, S., Montabana, E., Stanfield, R. L., ... & Wyatt, R. (2005). Structure of a V3-containing HIV-1 gp120 core. *Science*, 310(5750), 1025-1028.

Kirchherr, J. L., Hamilton, J., Lu, X., Gnanakaran, S., Muldoon, M., Daniels, M., Kasongo, W., Chalwe, V., Mulenga, C., Mwananyanda, L., Musonda, R.M., Yuan, X., Montefiori, D.C., Korber, M.T., Haynes, B.F., & Musonda, R. M. (2011). Identification of amino acid substitutions associated with neutralization phenotype in the human immunodeficiency virus type-1 subtype C gp120. *Virology*, 409(2), 163-174. DOI: 10.1016/j.virol.2010.09.031

Markham, R.B., Wang, W.C., Weinstein, A.E., Wang, Z., Munoz, A., Templeton, A., Margolick, J., Vlahov, D., Quinn, T., Farzadegan, H., & Yu, X.F. (1998). Patterns of HIV-1 evolution in individuals with differing rates of CD4 T cell decline. *Proc Natl Acad Sci U S A*. 95, 12568-12573. doi: 10.1073/pnas.95.21.12568