Does A Significant Change in CD4 Cell Count Affect The Number of Amino Acid Mutations In A Subject's Corresponding DNA Sequence

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Outline

- Markham paper focused on a segment of DNA in the V3 region.
- HIV replicates and mutates very often, which allows it to be unaffected by the body's immune response.
- This experiment was designed to show a connection between the change in a subject's CD4 cell count and their amino acid sequences.
- The results of this experiment showed evidence of the correlation between CD4 cell count and amino acid mutations.
- Mutations occurred most frequently between amino acids 38-42.
- Correlation between CD4 cell count and HIV virus mutations could be due to several reasons.

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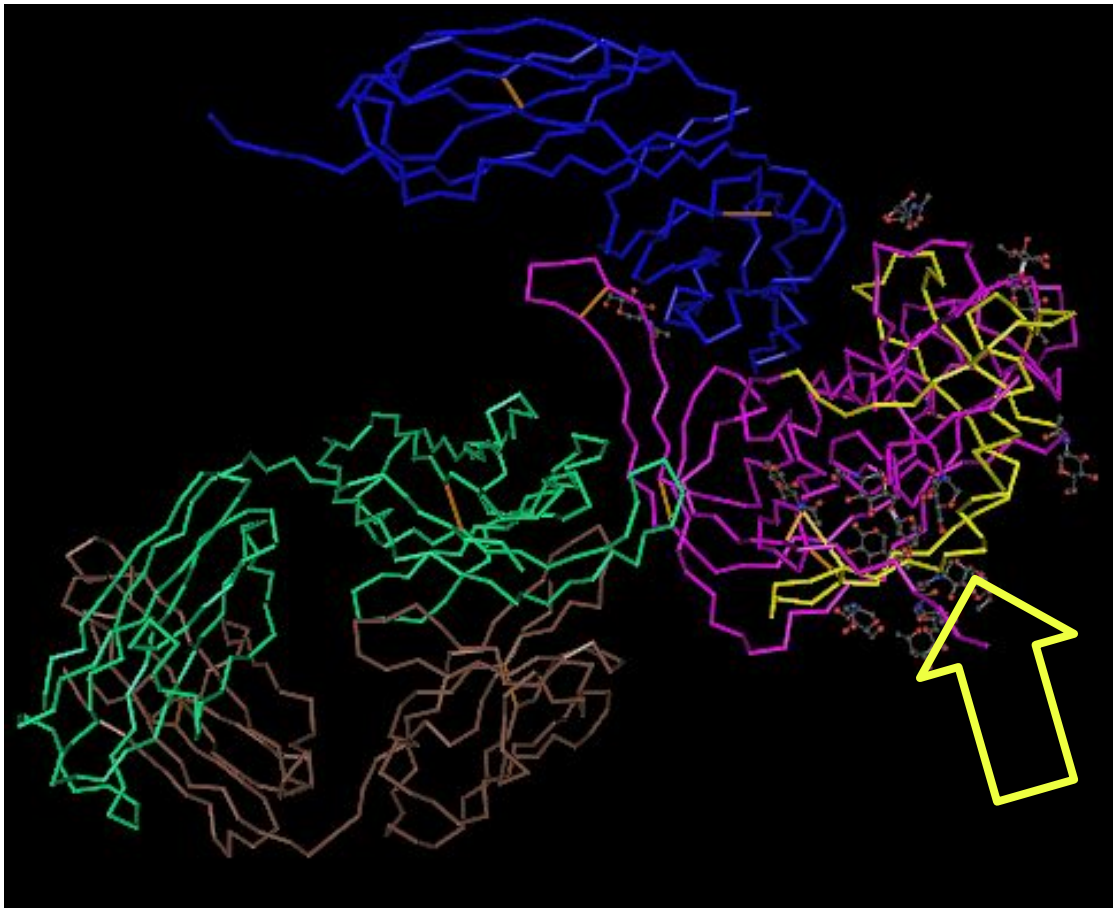
Markham Focused On The V3 Region

- Markham sequence that started with *EVV* ended with *HSS* was 95 amino acids long and was found within a 3D structure of an entire HIV virus copy.
- The highlighted section is what corresponds to the Markham sequence.



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Background

- CD4 cells are the white blood cells that fight infection for the body.
- These cells are the primary cells that are targeted by the HIV virus.
- The resilience of the HIV virus stems from its ability to mutate and multiply rapidly.
- Mutations occur due to two mechanisms:
  - Errors in replication.
  - Mutagenesis.
- More persistent viruses would have to mutate more often and have more mutations in their final form.

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Is There A Connection Between CD4 Cell Count And The HIV Virus?

- Question: Does a large decrease in CD4 count correlate to more mutational changes in the HIV virus?

- Purpose: To show a correlation between a subject's CD4 cell count and the number of mutations in their amino acid sequence.

A Decrease In CD4 Cell Count And An Increase In Amino Acid Mutations Was Suspected.

- Hypothesis #1: It was hypothesized that the rapid progressor subjects would have a larger amount of mutations, when comparing their first and last visits, than the nonprogressor subjects.

- Hypothesis #2: A large decrease in a subject's CD4 cell count would correlate with a large amount of amino acid mutations.

Subject's First and Last Visits Were Compared To Observe Mutational Differences In Amino Acids

- Clones from each subject's first and last visits were compared together.
  - Based on the idea that the virus sequences would change a large amount after the two year study.
- Rapid progressors subjects 10 and 4.
  - Less than 200 CD4 cells after study period.
- Nonprogressors subjects 12 and 13.
  - Greater than 650 CD4 cells after study period.

Several Processes Were Used to Obtain Data In This Experiment

- A multiple sequences alignment was conducted to observe trends in amino acid mutations.
  - Intent was to identify a trend of mutations which could be classified based on their characteristics. (i.e. polar uncharged→nonpolar)

- ClustalDist tool was used to measure the similarity between all the subjects, their visits, and clones.

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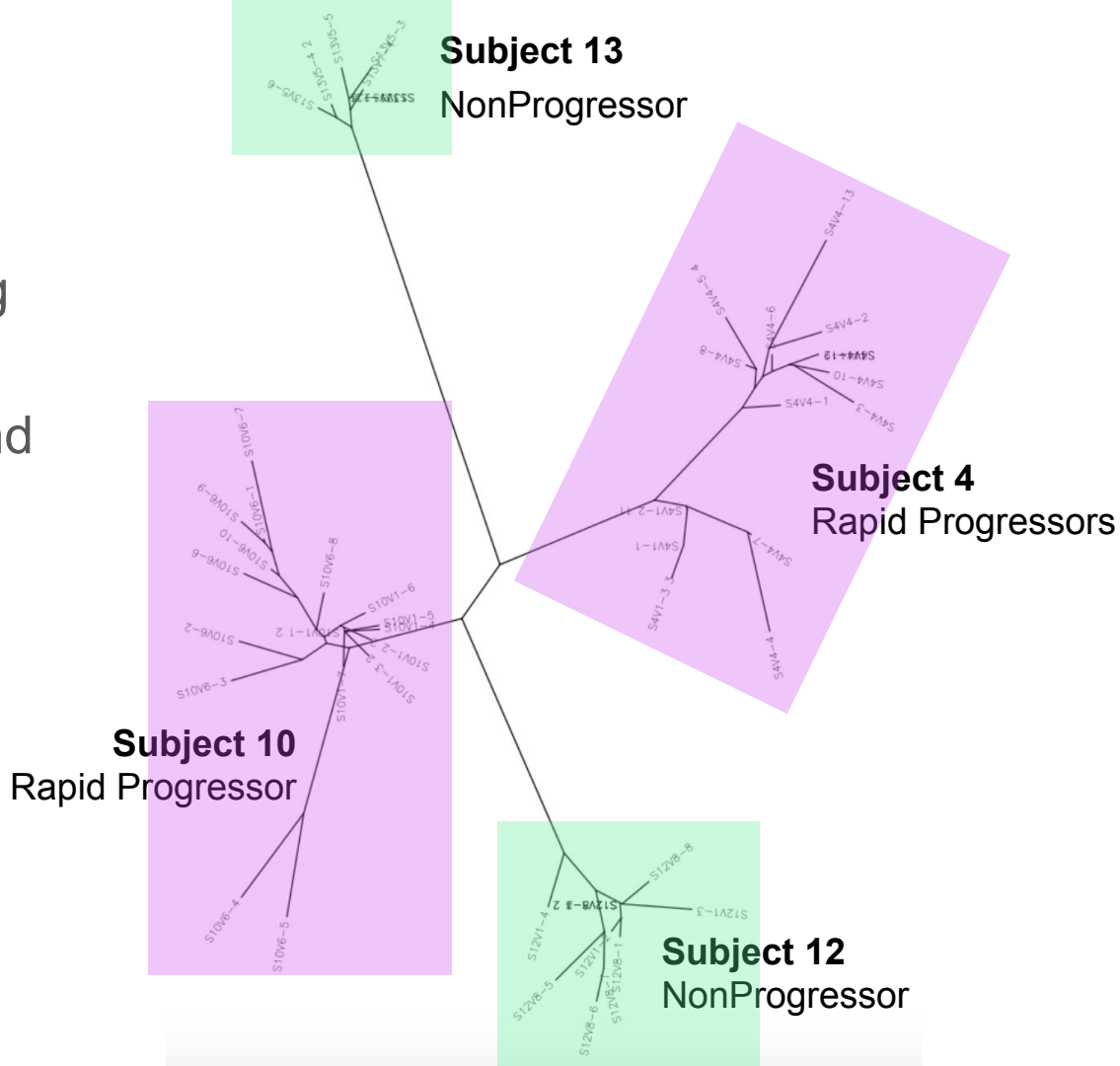
Analysis of Unrooted Tree

- Unrooted Tree presenting subjects 4, 10, 12, 13.
  - Including their first and last visits.
- Subjects 4 and 10 are rapid progressors.
- Subjects 12 and 13 are NonProgressors.



Results From ClustDist Support Both Hypotheses

Experimental Subjects

- Subject 13-visits 1 and 5
- Subject 12-visits 1 and 8
- Subject 10-visits 1 and 6
- Subject 4-visits 1 and 4

ClustalDist Results

- Subject 13: 0.021
- Subject 12: 0.053
- Subject 10: 0.116
- Subject 4: 0.117

Examination of Multiple Sequence Analysis Showed Correlation Between CD4 Cell Count and HIV Mutations

Amino Acid Mutations Between Subject's First and Last Visits

- Subject 4 → 20 mutation
- Subject 10 → 24 mutations

Rapid progressors

- Subject 12 → 11 mutations
- Subject 13 → 5 mutations

Nonprogressors

➔ There is a obvious difference in the amount of mutations between the rapid progressors and the non progressors. A T-test resulted in a value of 0.06.

➔ No consistently observable pattern of change was identified.

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Amino Acids 38-42 In the HIV Clones Studied Had the Largest Number Of Mutations

First Visits vs. Last Visits →	Subject 4	Subject 10	Subject 12	Subject 13
Number of mutations in amino acids 38-42 →	4	2	2	1

Amino Acids 38-42 In the V3 Region

- This is the region where the majority (>50%) of each subjects mutations occurred. Between amino acids 38-42 out of a 95 amino acid long sequence (V3 Region)
- Additionally, 30% of the mutations were either polar uncharged to hydrophobic or hydrophobic to hydrophobic.

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Our Hypothesis Was Proven Correct

- There is an obvious negative correlation between CD4 cell count and mutations in the HIV virus.
  - As CD4 cell declined, mutations in the HIV virus increased.

- As the body identifies the sequence of a virus it will develop antibodies to attack that region.
  - HIV virus will mutate to avoid antibodies.
  - The more adaptable an HIV virus is the more mutations it will have.
  - Thus, the more virulent strains in the rapid progressor subjects had the most mutations at the end of the study.

Amino Acids 38-42 Could Attach to Cytokines

- Amino acids 38-42 contained many polar amino acids.
- Suggests it could be a region that has a high affinity for polar cytokines.
    - Signalling protein.
- V3 region responsible for cell tropism (Response of cell to external stimuli).
- Cytokines allow for recognition of HIV virus.
    - Cytokines help HIV enter host cells.
- This would explain why this region has the most mutations because it must change its appearance to allow the virus to enter new cells as well as not be detected by antibodies.
    - (Yonezawa, Hori, Takaori-Kondo, Morita, & Uchiyama, 2001).

Summary

- The segment of HIV DNA studied in the Markham paper was the V3 region.
- HIV's ability to mutate and replicate allows it to avoid detection by host cell autoimmune response.
- The purpose of this experiment was to show a correlation between CD4 cell count change and mutations in the HIV virus DNA.
- The results of this experiment support our hypothesis the rapid progressors subject would have more mutations than the nonprogressor subjects.
- The region between amino acids 38-42 had the highest concentration of mutations.
- Two reasons can explain the correlation between CD4 cell count change and amino acid mutations in the rapid progressor subjects.

Acknowledgments

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References

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