dS/dN Ratios Do Not Provide A More Consistent Means of Comparison Between HIV-1 Progressor Groups

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Outline

1. **Analysis of Markham article and figures indicated there may be a better way to classify HIV-1 progressor groups.**
2. Data was reorganized and reanalyzed using s values, theta values, and the formation of comparative trees.
3. Statistical analysis of data showed no significant difference between subjects.
4. Questioning utility of dS/dN ratio modeling may explain insignificant results.
5. Further research involves reanalysis of Markham data using slope of nucleotide difference and slope of divergence.

Patterns of HIV-1 evolution in individuals with differing rates of CD4 T cell decline.

Markham, R. B., Wang, W., Weisstein, A. E., Wang, Z., Munoz, A., Templeton, A., . . . Yu, X. (1998). Patterns of HIV-1 evolution in individuals with differing rates of CD4 T cell decline. *Proceedings of the National Academy of Sciences, 95*(21), 12568-12573. doi:10.1073/pnas.95.21.12568

- Progression of the disease HIV was analyzed and correlated with CD4 (T-cell) counts.

- dS/dN values were computed for each subject's consensus strain.

- Subjects were categorized into three groups: Rapid progressor, moderate progressor, and nonprogressor. *(Markham et. al, 1998)*

What is dS/dN value?

- Non synonymous mutations (dN) are nucleotide changes that lead to the coding of a different amino acid.
- Synonymous mutations (dS) are nucleotide changes that do not lead to the coding of a different amino acid.
- These ratios were formed for each consensus strain for all subjects.
    - A single averaged ratio was taken from all consensus strains.
    - Each strain was compared to a subsequent strain to observe differences.
- The smaller the ratio, the larger amount of non synonymous mutations.

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Markham's data was used to create a purpose for this experiment.

- A study was designed to analyze the dS/dN ratios of each subject.
  - The study involved the usage of Markham's dS/dN values, subjects, and classifications (Rapid progressor and nonprogressor).
- The purpose of this study was to analyze the dS/dN ratios of each subject and use this to show significant difference between the rapid progressors and nonprogressors.

Preparing to reanalyze Markham's data cont.

- Hypothesis #1: It was hypothesized that smaller dS/dN ratios would correlate with the newly classified rapid progressor subjects.
    - Null Hypothesis #1: It was hypothesized that larger dS/dN ratios would correlate with rapid progressor subjects.
- Hypothesis #2: It was hypothesized that newly classified rapid progressor subjects would have higher theta values due to a higher level of genetic diversity.
    - Null Hypothesis #2: It was hypothesized that the rapid progressors would have lower theta values due to their lower level of genetic values.

Reorganizing subjects and dS/dN values.

- dS/dN ratios were ranked in increasing order.
- Two groups of four subjects were selected from the two ends of the spectrum: Nonprogressor and rapid progressor.
- Non-progressor group:
  - Subjects: 4, 9 , 11, 14
  - The nonprogressor group was selected so that all subjects had a dS/dN value of 0.
- Rapid Progressor group:
  - Subjects: 7, 5, 2, 13
  - The rapid progressor subjects had a dS/dN value ranging from 1.3-3.5.

Markham's subjects were reorganized within existing classifications.

Rapid Progressor (Subject #)	Moderate Progressor (Subject #)	Non-progressor (Subject #)
4	**7**	**2**
10	8	12
11	**14**	**13**
15	**5**	
3	**9**	
1	6	

Table 1: Markham's classification of subjects based on CD4 cell counts.

Rapid Progressor (Subject #)	Nonprogressor (Subject #)
7	4
5	9
2	11
13	14

Table 2: Reclassification of subjects based on dS/dN value.

(Markham et. al, 1998)

Theta values and unrooted/rooted trees were calculated to compare results.

- Theta values were calculated from the number of position where there was at least one nucleotide difference across all that subject's clones (S value).
  - This value was calculated to show a significant difference between rapid progressors and nonprogressors.
  - Rapid progressors presumed to have higher theta values (more genetic diversity) due to the increased presence of nonsynonymous mutations.
- Unrooted and rooted trees were generated for the purpose of presenting visual evidence of the significant difference between rapid progressors and nonprogressors in terms of genetic similarity.

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Statistical results showed no correlation between dS/dN values and subject groups.

- The theta values calculated for each subject were not the expected numbers for those subjects in the nonprogressor and rapid progressor groups.
  - Ex. Subject 4 (NP-group): 15.8 Subject 5 (RP-group): 13.6
- The theta values calculated for each subject were averaged for both the nonprogressor and rapid progressor groups.
  - The resulting two theta values were measured for statistical significance using a t-test and no statistically significant difference was found.
  - P-value (significance= <0.05) of 0.146

There is no significant difference between Θ values of reclassified groups.

Subject #	2	5	7	13
"S" Value	36	58	55	25
"Θ" Value	9.6	13.6	12.7	6.6

Table 3: Calculated s-values and theta values of reclassified non progressor subjects.

Average "Θ" Value: 10.63

Subject #	4	9	11	14
"S" Value	70	69	41	77
"Θ" Value	15.8	14.6	10.2	15.7

Table 4: Calculated s-values and theta values of reclassified rapid progressor subjects.

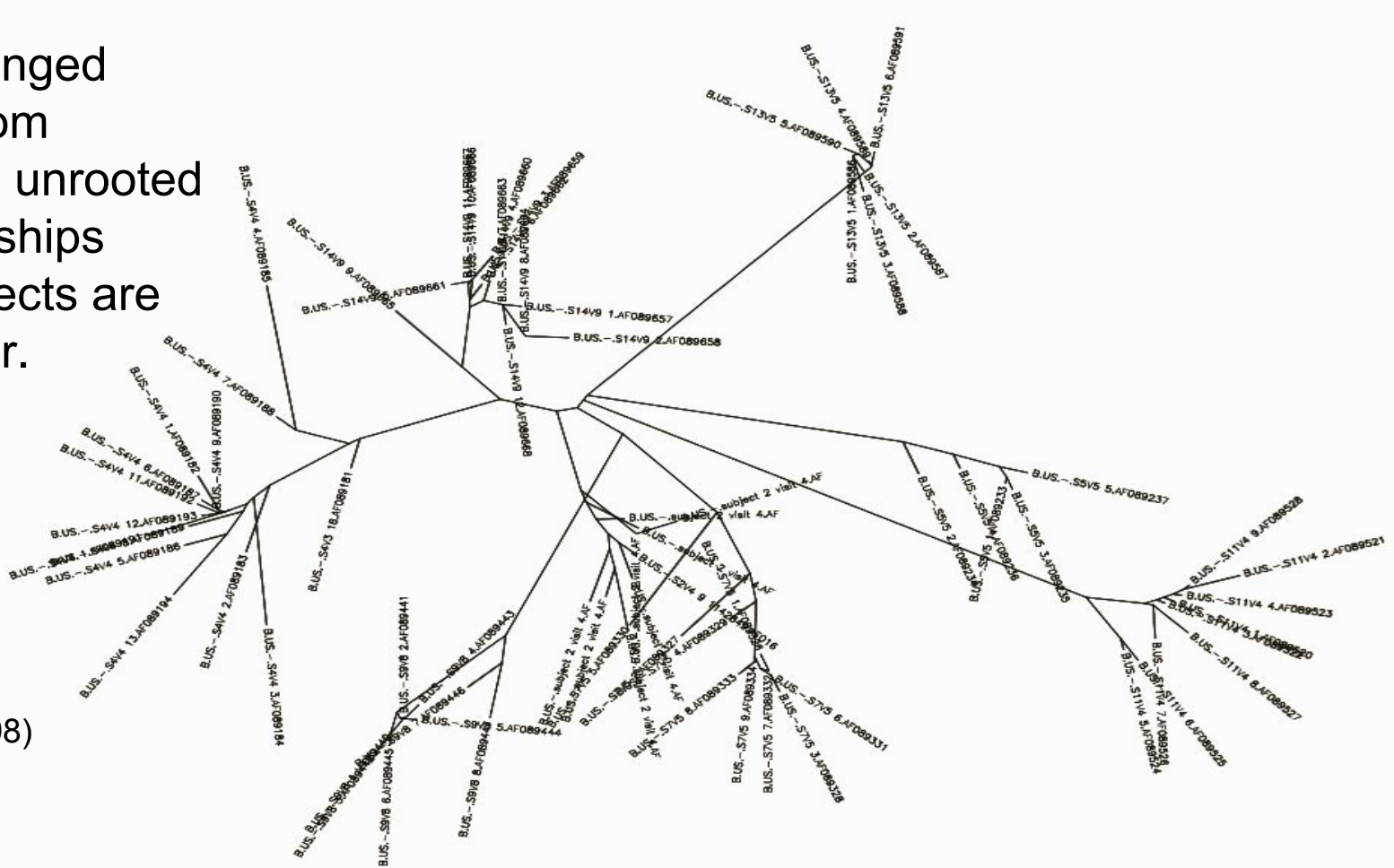
Average "Θ" Value: 14.08

Statistical Significance (<0.05): P-Value: 0.146

*Θ Values calcultaed using mathisfun.com (Subramaniam, 1998)

Interpretation of rooted and unrooted trees.

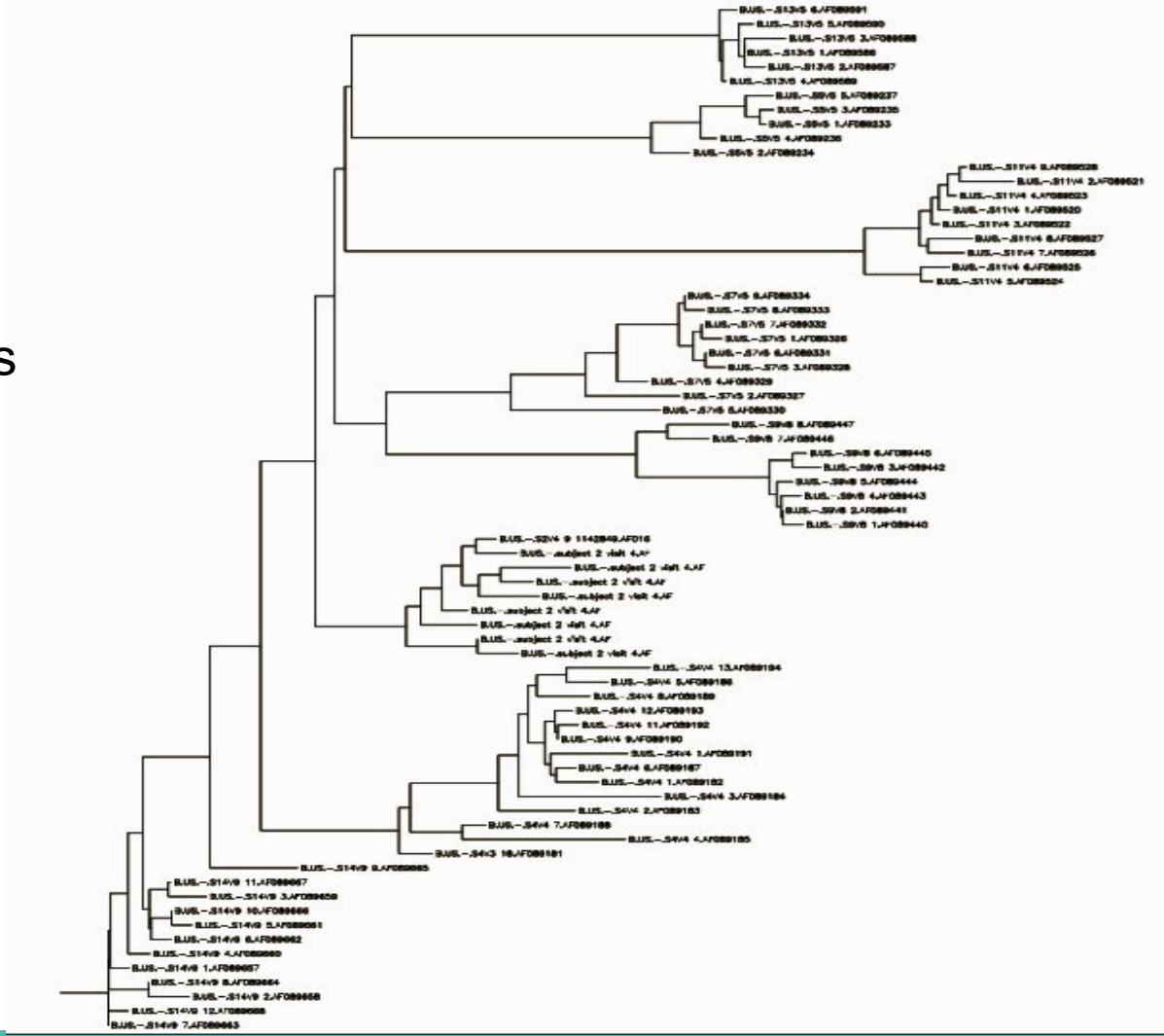
Figure 1: Arranged sequences from subjects in an unrooted tree. Relationships between subjects are difficult to infer.



(Subramaniam, 1998)

Interpretation of rooted and unrooted trees.

Figure 2: Arranged sequences from subjects in rooted tree format demonstrating non-distinct relationships between subjects classified in new groups.



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The Relationship between dN/dS and Scaled Selection Coefficients

Spielman, S. J., & Wilke, C. O. (2015). The Relationship between dN/dS and Scaled Selection Coefficients. Molecular Biology and Evolution, 32(4), 1097-1108. doi:10.1093/molbev/msv003

- dS/dN ratio modeling assumes constant selective pressures over time between subjects.
- Confounding variables may cause differences in mutation rates.
- dN/dS ratio threshold =1 is highly sensitive to modeling assumption violations.

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Reclassification based on genetic diversity or divergence may support initial hypotheses.

- Re-analyzing subjects based upon slope of change in nucleotide difference or slope of divergence may provide a more accurate method of analysis.
- Groups would still be classified as rapid progressor and nonprogressor, however dS/dN ratios would no longer be important to our investigations.
- T-test analysis of groups based off of diversity/divergence may show significant results.
    - S-values and theta values would be incorporated into this test.

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Acknowledgements

Dr. Dahlquist

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References

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