

Rapid Progressors and Moderate Progressors Should be Categorized in the Same Group, Despite Markham et al. Classifying Them in Different Groups

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Outline

- **Markham et al. (1998) conducted a study on HIV-1 evolution by looking at patterns over time.**
- **Markham et al. (1998) placed rapid and moderate progressors in different groups due to CD4 cell decline from HIV-1.**
- **However, no significant difference was found in their study.**
- **Should rapid and moderate progressors be categorized in the same group since they have no significant difference?**
- **We collected data from the first and two year visit of the rapid and moderate progressors.**
- **We calculated theta and performed a t-test which showed that rapid and moderate progressors should be in the same group.**

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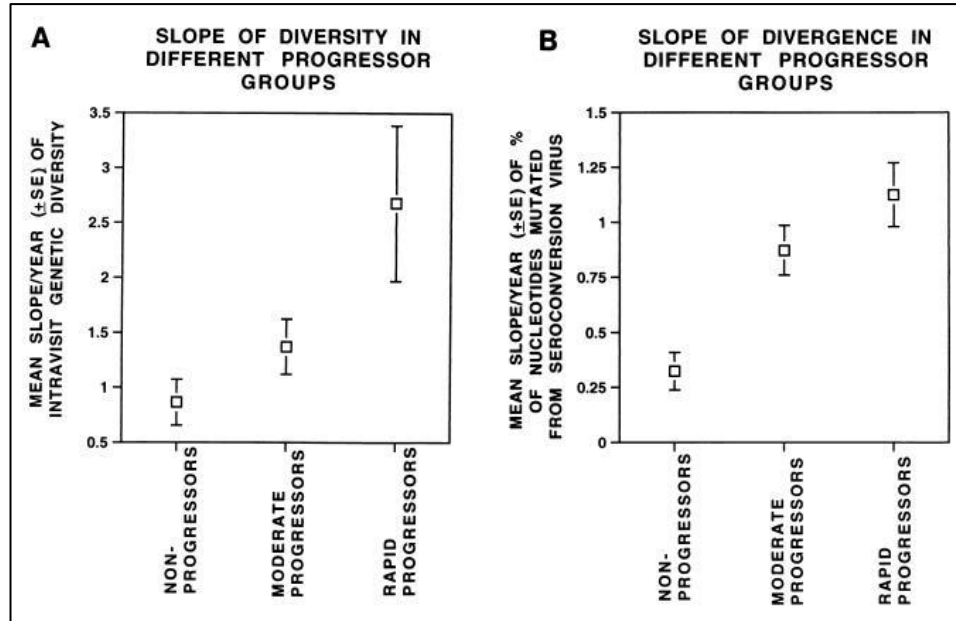
- **Human Immunodeficiency Virus (HIV) has a much higher mutation rate than its host cell**
- **Markham et al. studied 15 subjects within a four year observation period**
- **Clones from each visit were sequenced**
- **Diversity and divergence were analyzed over time between and within subjects**

Markham et al. separated rapid and moderate progressors in different groups due to CD4 cell decline

Markham et al. (1998) categorized their subjects into three categories

- **Rapid progressors: obtained a level of fewer than 200 CD4 cell count within the first two years of seroconversion**
- **Moderate progressors: declined to 200-650 cell count during the 4 year observation period**
- **Nonprogressors: maintained a level of CD4 cells above 650**

Rapid and moderate progressors had no statistical difference regarding diversity and divergence



Markham et al. (1998) *Proc Natl Acad Sci U S A*. 95:21

The Main Question

Should rapid and moderate progressors be classified in the same group?

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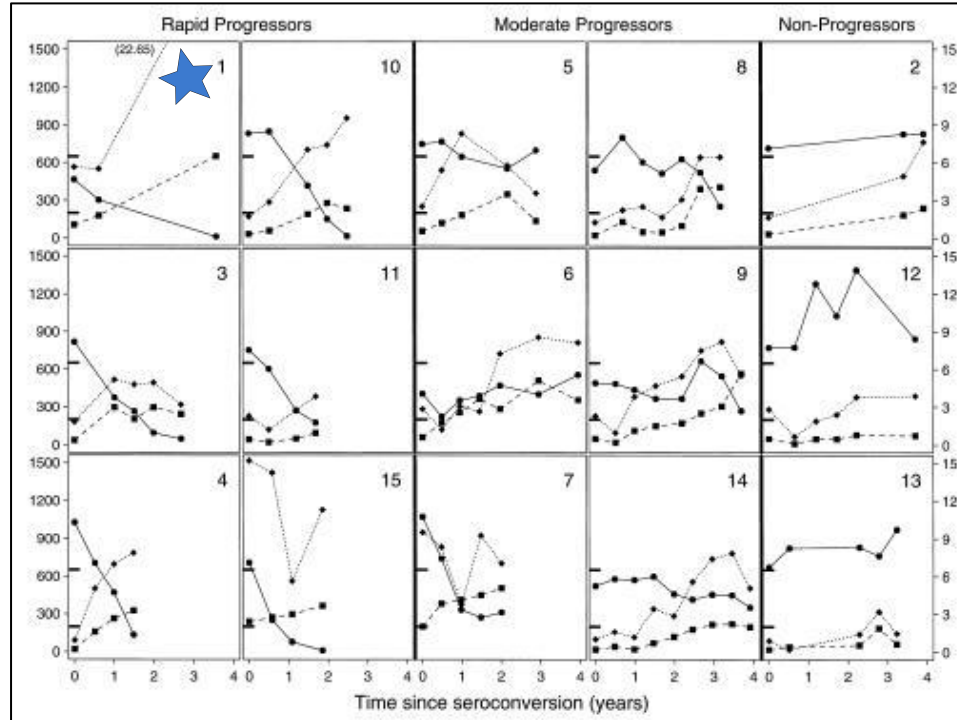
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Subjects 4 and 7 disregarded from this study due to inconsistency

Table 1. Summary data on 15 seroconverters

Subject	No. of observations	Baseline			Annual rate of CD4 T cell decline	Slope of change in intravisit nucleotide differences per clone per year	Slope of divergence (% nucleotides mutated from baseline consensus sequence per year)	Median dS/dN
		CD4	Median intravisit nucleotide differences among clones	Virus copy number (×10 ³)				
Rapid Progressor								
Subject 4	4	1,028	0.90	6.8	−593	4.64	2.09	0.0
Subject 10	5	833	1.71	99.3	−363	3.16	1.00	0.2
Subject 11	4	753	2.27	62.2	−363	1.11	0.32	0.0
Subject 15	4	707	15.16	171.0	−362	−2.94	0.68	0.7
Subject 3	5	819	1.82	302.5	−294	0.53	0.74	1.0
Subject 1	3	464	5.64	307.6	−117	5.10	1.55	0.3
Moderate Progressor								
Subject 7	5	1,072	2.27	317.6	−392	−0.79	1.35	1.3
Subject 8	7	538	1.24	209.0	−92	1.68	1.16	0.5
Subject 14	9	523	1.00	50.9	−51	1.69	0.60	0.0
Subject 5	5	749	2.50	260.6	−41	0.06	0.50	1.4
Subject 9	8	489	9.49	265.0	−11	1.58	1.21	0.0
Subject 6	7	405	2.82	321.4	52	1.92	0.82	0.4
Nonprogressor								
Subject 2	5	715	1.64	21.6	30	1.32	0.49	1.8
Subject 12	6	772	2.80	5.1	44	0.62	0.13	0.9
Subject 13	5	671	0.87	1.7	53	0.53	0.28	3.5

Data was observed from first and 2 year visit
Subject 1 was disregarded due to lack of visits

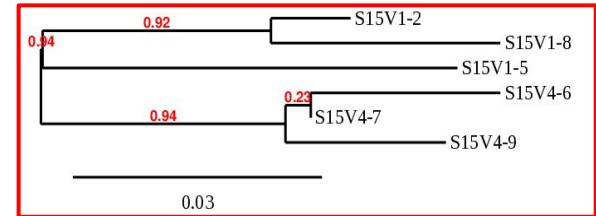
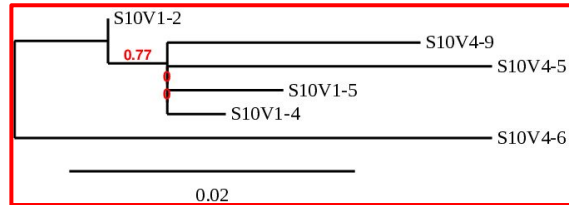
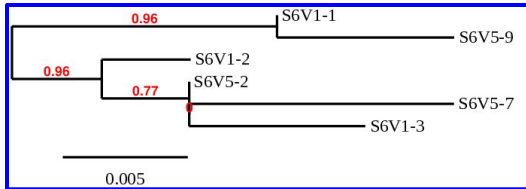
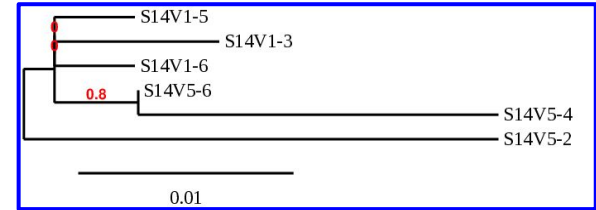
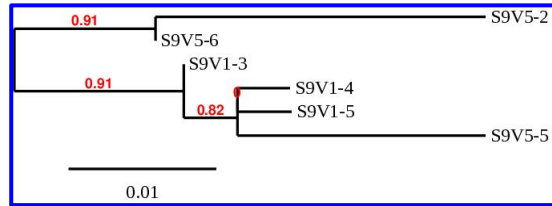
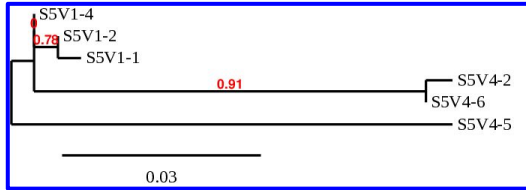
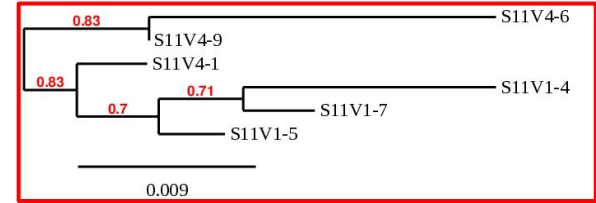
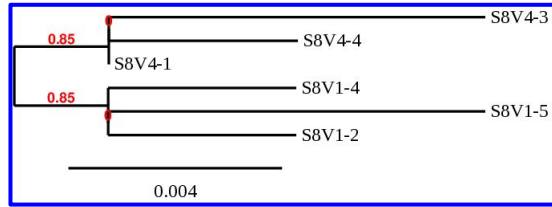
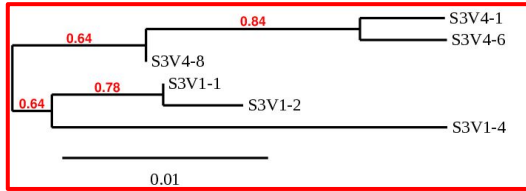


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Three clones from each of the chosen subjects were randomly selected

- **Out of all the visits, subject 6 had a maximum of three clones from the first visit**
- **Therefore, to keep the data uniform, three clones were chosen at random for the remaining subjects for the first and second year visit.**

No Pattern Observed in Phylogenetic Trees of Subjects



 = Rapid = Moderate

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Theta was calculated using multiple sequence alignment of the clones

- Clones from each subject were aligned in CLUSTAL format
 - First visit and two year visit were aligned separately
- Theta calculates average pairwise genetic distance
 - S is the number of nucleotide differences
 - Harmonic sum was found using a harmonic sum calculator
- Compared theta values for each subject from the 1st visit and the 2 year visit

$$\theta = \frac{S}{\sum_{i=1}^n \frac{1}{j}}$$

t-test was used to determine if rapid and moderate progressors should be in the same group

- **Theta differences between 2nd year and first visit were used for statistical analysis**
- **t-Test is used to test the null hypothesis that the means of two populations are equal**
 - **Null hypothesis: Are the means of the rapid and moderate progressors equal?**

Rapid	Moderate
-0.55	3.82
5.46	0.55
0	-0.54
-8.7	4.36
	2.18

F-test showed that the variances were unequal between rapid and moderate progressors

- T-test assumes that the variances are unequal between the two tests
 - Therefore, a F-test was conducted beforehand
- F-test is used to test the null hypothesis that the variances of two populations are equal
 - If $F > F\text{-critical one-tail}$, then we can reject the null.
 - Since $7.81 > 6.59$, we can **reject** the null-hypothesis.
- The variances of the two populations are unequal.
 - Therefore, we can proceed with the t-test.

	Variable 1	Variable 2
Mean	-0.9475	2.074
Variance	34.071025	4.36028
Observations	4	5
df	3	4
F	7.813953462	
P(F<=f) one-tail	0.03784583131	
F Critical one-tail	6.591382116	

t-test showed no significant difference between the two groups

- If $t_{\text{Stat}} > t_{\text{Critical two-tail}}$, the null hypothesis is rejected
 - $-0.98 > 2.77$ is not true, therefore, it is **not** rejected
- The observed difference between the rapid and moderate progressors is **not statistically different**
- And therefore, should belong in the **same group**

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	Variable 2
Mean	-0.9475	2.074
Variance	34.071025	4.36028
Observations	4	5
Hypothesized Mean Difference	0	
df	4	
t Stat	-0.9860391174	
P(T<=t) one-tail	0.1899683194	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.3799366387	
t Critical two-tail	2.776445105	

Variance difference and sample size in t-test could explain why there is no significant difference

- Even though no significant difference was observed, there is a 8-fold difference in variance between rapid and moderate progressors as well as a small sample size.
- These factors could have led to the two groups being non-significant.

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Future Research

- **Markham et al. could repeat the study by using different factors that affect the p value of the statistical test.**
- **Including, but not limited to:**
 - **Larger cohort size**
 - **Consistent visits**
 - **Diverse group of subjects**

Acknowledgements

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