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A BAYESIAN STATISTICAL ANALYSIS OF HTLV-II EVOLUTIONARY RATES IN INTRAVENOUS DRUG USERS COMPARED TO ENDEMICALLY INFECTED TRIBES

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Abstract

Human T-Lymphotropic Virus Type II (HTLV-II) is a retrovirus that has shown high genetic stability, and low sequence diversity. This low diversity is thought to arise from HTLV-II's mechanism of replication. HTLV-II is present in Europe and the Americans mainly among Intravenous drugs users (IDUs) and has been show to be endemic in a number of South American and African tribes. Within the former, needle sharing has been identified as the main mechanism of transmission (horizontal transmission), whereas in the latter, the virus is transmitted from mother to child via breast milk (vertical transmission). Previous authors have identified different rates of HTLV-II evolutionin viruses transferred among IDUs and viruses transferred between mother and child in endemically infected tribes. Here, we test whether HTLV-II evolution is dependent on mechanism of transmission. Using updated global HTLV-II sequences and a flexible Bayesian Markov chain Monte Carlo (MCMC) coalescent approach, our results show clearly, in contrast to published data, that HTLV-II evolves at a constant rate, regardless of mechanism of transmission.

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A Bayesian Statistical Analysis of HTLV-II Evolutionary Rates in Intravenous Drug Users Compared to Endemically Infected Tribes

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Human T-Lymphotropic/Leukemia Virus Type I (HTLV-I) and Type II (HTLV-II) are oncogenic retroviruses and were the first two human retroviruses to be discovered (Poiesz, 1980; Kalyanaraman 1982). Like all viruses belonging to the family *retroviridae*, HTLV-I and HTLV-II are single-stranded RNA (ssRNA) viruses that utilize reverse transcriptase (ssRNA-RT), an RNA-dependent DNA polymerase, to generate a DNA intermediary, which can integrate into the host genome as a provirus (Blattner, 2000). In contrast to HIV, another retrovirus, HTLV-I and HTLV-II have shown high genetic stability and low pathogenicity (Gessain, 1992). The low diversity of HTLV is thought to arise from its mechanism of replication. As a retrovirus, HTLV utilizes the host cell DNA polymerase, which contains a proofreading exonuclease, for replication. This accounts for the high fidelity of copies of the parent provirus in daughter cells subsequent to mitosis (Lemey et al., 2005). Unlike HIV, a similar retrovirus, HTLV does not appear to exploit it's capacity to generate considerable sequence diversity through it's highly error prone reverse transcriptase. Furthermore, replication appears to be maintained through clonal expansion of the host cell (Wattel et al., 1995).

HTLV-I has been connected with Adult T-Cell Leukemia/Lymphoma (ATL) and Tropical Spastic Paraparesis/HTLV-I associated Myelopathy (TSP/HAM). HTLV-II remains an "orphan" virus, with no true disease association, though some evidence does exist to suggest its involvement with certain neurological diseases (Blattner, 2000; Hall et al., 1994; Hall et al., 1996, Murphy et al., 1997).

There are four identified subtypes of HTLV-II (Hall et al., 1992, Ishak et al., 2001). The two main subtypes, IIa and IIb, are present in the US (Hjelle et al. and Takahashi et al., 1993), Europe, Vietnam (Fukushima et al., 1995), South America (Lewis et al., 2000) and several Native American tribes (Dueñas-Barajas et al., 1993; Switzer et al., 1995; Biggar et al., 1996). The HTLV-IIc subtype was recently isolated from Kayapo Indians in the Amazon Region of Brazil (Ishak, 2001) and the occurrence of the IId subtype has been confirmed in a Mbuti Efe Pygmy (Vandamme et al., 1998).

HTLV-II is assumed to have an ancient African origin. Previous studies suggested that the original HTLV-II virus migrated with humans from Asia over the Bering land bridge some 15,000 to 35,000 years ago (Lairmore et al., 1990; Pardi et al., 1993; Neel et al., 1994; Biggar et al., 1996; Suzuki et al. and Gojobori et al., 1998). However, the extent to which HTLV genetic variation is representative of mammalian migration patterns is unknown; such calibrations depend heavily on the anthropologic accuracy of the dates being used (Lemey et al., 2004).

In Europe and the Americas, HTLV-II is mainly present among intravenous drug users (IDUs). It has also been shown to be endemic in a number of South American and African tribes (Lairmore et al., 1990; Goubau et al., 1992; Hjelle et al. and Dueñas-Barajas et al., 1993; Gessain et al. and Switzer et al., 1995; Biggar et al., 1995; Blattner,

2000). Within endemically infected tribes, the virus is transmitted from mother to child via breast milk (Van Dyke, 1995), whereas in IDUs, needle sharing is the primary means of HTLV-II transmission (Lee et al., 1989; Black, 1997; Blattner, 2000).

Different rates of HTLV-II evolution have been identified for viruses transferred among IDUs (horizontal transmission) and viruses transferred among people in endemically infected tribes (Vertical transmission) (Salemi et al., 1998 and 1999; Lemey et al., 2005). The differences in evolutionary rate are particularly apparent in the Long Terminal Repeat (LTR) region of the HTLV-II genome, which is essential for viral integration and regulation of gene expression (Blattner, 2000). In 1999, Salemi *et al.* suggested that different rates of substitution exist for the HTLV-II virus in IDUs vs. endemically infected tribes (END's). To estimate these evolutionary rates, each study has assumed a molecular clock, which assumes that rate variations on each branch of a phylogeny are not independent of each other. These models can assume that either nucleotide substitutions accumulate at a fixed rate (constant) through time (strict clock) or that rates may vary among lineages in a controlled manner, as previously defined by a clock model (relaxed clock). To satisfy the assumption of the molecular clock, many of these studies have been forced to discard sequence data that violate the assumption of the molecular clock (Yanagihara et al., 1995; Salemi et al., 2000; Van Dooren et al., 2001; Meertens and Gessain., 2003; Meertens et al, 2003). Additionally, many of these studies ignore uncertainty associated with the anthropological calibrations, which can also influence the evolutionary rate estimation (Lemey et al., 2004).

Here, we test whether HTLV-II evolves at a different rate during horizontal versus vertical transmission, using a flexible Bayesian Markov chain Monte Carlo (MCMC) coalescent approach and an updated data set of global HTLV-II sequences. In contrast to the published data, our results show clearly that HTLV-II evolves at a constant rate, regardless of the mechanism of transmission.

MATERIALS AND METHODS

Sequence Data. We collected all HTLV-II LTR sequences available from GenBank (Table 1) as of October 2009 in accordance with the specifications previously defined by Salemi et al., 1999, which selected HTLV-II sequences representing the LTR region of the HTLV-II genome-"the largest dataset available for the fragment corresponding to nucleotides 315-706 of the HTLV-IIa Mo isolate" (Salemi et al., 1999). We created three data sets: IIend was composed of 30 IIa and IIb strains isolated from different Amerindian tribes, the Efe2 IId strain from a Mbuti Efe Pygmy (Vandamme et al., 1998) and 6 IIc strains recently isolated from Brazil (Ishak et al., 2001; Catalan-Soares et al., 2005); IIidu was composed of 68 IIa and IIb strains isolated from IDUs; and New_All_Sequences, was composed of the all of the sequences from both the IIend and IIidu alignments. All sequences were aligned using ClustalX version 2.0, the graphical interface version of ClustalW (Larkin et al., 2007) after preparation using Se-Al v2.0a11 (Rambaut, 2008).

Temporal Signal. The availability of nucleotide and amino acid sequences of virus populations, sampled at different points in time has cultivated new statistical methods that utilize this temporal information. If a statistically significant number of genetic differences have accumulated within this time period, the population is considered to be a measurably evolving population (MEP) and these statistical methods, which calibrate the molecular clock model by using the ages of each sampled sequence, may be employed (Drummond et al., 2003). RNA viruses evolve rapidly and thus are considered to be MEPs. Previous work, however, has suggested that the HTLV-II virus evolves too slowly to allow tip-calibration (Salemi et al., 1999; Lemey et al., 2005). These studies instead suggest that an internal calibration point, such as the dispersal of the virus across the Bering Land Bridge, would be a better method to calibrate the molecular clock.

Our Bayesian MCMC approach requires several assumptions. We assume an evolutionary model, a rate of evolution, and a coalescent, or demographic prior. The following paragraphs describe how we

identify and select the best-fitting priors for our analyses. All analyses were performed using the Bayesian phylogenetic inference package, BEAST (Drummond and Rambaut 2007).

Evolutionary Model. For each data set, evolutionary models were selected using MODELTEST v3.7 (Posada et al., 1998) under the Akaike Information Criterion (AIC). The models selected for each data set are provided in Table 2.

Molecular Clock Rate. We tested whether the viruses were evolving according to a molecular clock. We first used the temporal information inferred from sampling times of each viral sequence; For each data set, we assumed both a strict and relaxed molecular clock model (Drummond et al., 2006), which allows the evolutionary rate to vary among lineages. The two models were then compared using Bayes Factors (Suchard et al., 2001) to determine which model was more appropriate for each data set. These models were then compared to a comparable isochronous model, to determine whether tip dating was informing the clock.

Constant V. Bayesian Skyline Coalescent Prior. As the demographic history of the sequences was unknown, it was also necessary to perform a test to determine the best fitting coalescent prior for our analyses. To perform this test, we compared a constant population size coalescent prior with a flexible (Bayesian skyline plot; Drummond et al 2005) coalescent prior.

For each analysis, two runs of 100 million generations were performed, with samples drawn from the posterior every 10 thousand generations. The first 10% of runs were discarded as burn-in and the remainder were combined. Convergence to stationarity was evaluated using Tracer v 1.4 (Rambaut and Drummond, 2007). Maximum Clade Credibility (MCC) genealogies were created using TreeAnnotator (Drummond and Rambaut 2007) and visualized in FigTree (Rambaut, 2008).

Strict Rate Comparison. Due to lack of temporal information, it was not possible to calibrate the rate of evolution with the ages of the sequences as prior information. We therefore constrained the molecular rate with two previously determined rates that had been generated using internal calibrations. Assuming a strict clock model, we sampled the evolutionary rate between 1.2_E^{-7} and 1.0_E^{-3} nucleotides/site/year, which is the full range of possible rates as found in previous studies (Salemi et al., 1998 and 1999; Lemey et al., 2005). Next, we sampled the clock model between an upper bound of 1.1_E^{-6} nucleotides/site/year and a lower bound of 1.2_E^{-7} nt/site/yr, which is the rate that has been attributed to the slowly evolving sequences belonging to endemically infected tribes (Salemi et al., 1999; Lemey et al., 2005). It should be noted that this test was only performed on the dataset New_All_Seuqneces, the purpose of which, was to determine whether the slower rate was a better fit for the full phylogeny than a rate that included all previously identified rates. Tests were performed with both a constant population size coalescent prior and a flexible coalescent prior as described above. Bayes factors (Suchard et al., 2001) were used to discriminate between these models.

Specific Delta Model. Finally, we used a new parameter, the Specific Delta Model (SDM), to further investigate whether the IDU sequences were evolving at a different rate than the END sequences. Whereas the relaxed clock model allows for a distribution of evolutionary rates across the tree, the SDM has the advantage both of having many fewer parameters and of providing an explicit test of whether a different rate of evolution exists in a specified subset of the data (Harrison et al, in prep). The relaxed clock model works by estimating two different parameters to describe variation in substitution rate within an alignment. The ulcdMean rate (μ) is an average across all branches of a phylogeny, weighing each branch equally, regardless of length in evolutionary time. Alternatively, the meanRate (μw) is weighted, taking branch length and evolutionary time, into consideration before estimating an average. In most circumstances, μ and μw will be equal, assuming rate variation is distributed randomly throughout a phylogeny. The alignment New_All_Sequences includes sequences sampled from populations identified previously (Salemi et al., 1998 and 1999; Lemey et al., 2005) as evolving at differing rates. In this situation μ and μw might differ. To allow for such a difference, Harrison et al (in prep) developed a model that allows for a subset of lineages (here, the IDU lineages) to evolve at a rate different from that of the rest of the tree. Bayes factors (Suchard et al., 2001) were used to determine whether models including the parameter are a better fit for the data than models without the parameter.

Table 1. Information about the sequences used in this study.

Name	GenBank E#	Year	Subtype	Reference
Iiend				
WY018	D82957	1991	IIb	Miura et al, 1997
WY100	D82958	1991	IIb	Miura et al, 1997
SEM1050	U10263	1994	IIb	Switzer et al., 1995
2C11521	D82956	1992	IIb	Miura et al, 1997
WYU2	U12794	1995	IIb	Switzer et al., 1995b
CH13504	D82959	1991	IIb	Miura et al, 1997
SEM1051	U10264	1994	IIb	Switzer et al., 1995
AGPUEB (PUEB.AG)	U10261	1994	Iia	Switzer et al., 1995a
FH	L37133	1995	IIb	Eiraku et al., 1995
SC	L37140	1995	IIb	Eiraku et al., 1995a
WY (BLAST: WV)	L37129	1995	IIb	Eiraku et al., 1995a
JD	L37137	1995	IIb	Eiraku et al., 1995a
G12	L11456	1993	IIb	Pardi et al., 1993
WYU1	U12792	1995	IIb	Switzer et al., 1995b
2C3821	D82954	1992	IIb	Miura et al, 1997
2C2517	D82953	1992	IIb	Miura et al, 1997
2C5505	D82955	1992	IIb	Miura et al, 1997
2C1801	D82952	1992	IIb	Miura et al, 1997
KAY73	L42509	1996	IIa	Vandamme et al., 1998
KAY2	U19109	1995	IIa	Vandamme et al., 1998
KAY1	U19109	1995	IIa	Vandamme et al., 1998
KAY139	L42508	1996	IIa	Vandamme et al., 1998
PUEB.RB	U10262	1994	IIa	Switzer et al., 1995a
NAV.DS	U10257	1994	IIa	Switzer et al., 1995a
DSA	L37132	1995	IIa	Eiraku et al., 1995
Efe2	Y14365	1995	IId	Vandamme et al., 1998
Iiidu				
JL_JAN	L77241/L77242	1996	IIb	Vallejo et al., 1996
HCM6	AF020632	1997	IIb	Fukushima et al., 1995
Ag1	AF020635	1997	IIb	Fukushima et al., 1995
Gu	X89270	1995	IIb	Salemi et al., 1996
I_OG	Y09154	1997	IIb	Vandamme et al., 1998
I_IT	Y09151	1997	IIb	Vandamme et al., 1998
I_OV	Y09155	1997	IIb	Vandamme et al., 1998
I_AM	Y09149	1997	IIb	Vandamme et al., 1998
SPAN129	U10265	1994	IIb	Switzer et al., 1995a
HCM1	AF020627	1997	IIb	Fukushima et al., 1995
FUC	L37135	1995	IIb	Eiraku et al., 1995
PAR	L37145	1995	IIb	Eiraku et al., 1995
HCM4	AF020630	1997	IIb	Fukushima et al., 1995
VIET35	U72533	1996	IIb	Vandamme et al., 1998
JG (b)	L06857	1992	IIb	Vandamme et al., 1998
GAR	L37136	1992	IIb	Takahashi et al., 1993
I_GI	Y09153	1997	IIb	Vandamme et al., 1998
I_EA_I_EC	Y09152-Y09150	1997	IIb	Vandamme et al., 1998

BF_RC	L77235-L77236	1996	IIb	Vandamme et al., 1998
SPAN130	U10266	1994	IIb	Switzer et al., 1995a
VIET19_32	U72525/U72532	1996	IIb	Lin et al., 1997
HCM2	AF020628	1997	IIb	Fukushima et al., 1995
324_	L77243	1996	IIb	Vallejo et al., 1996
RVP	L77244	1996	IIb	Vallejo et al., 1996
DP_NY185	L7725/U10259	1994	IIb	Switzer et al., 1995a
ITA47A	U10254	1994	IIb	Switzer et al., 1995a
AA_JA	L77238-L77239	1996	IIb	Miura et al, 1997
CAM	L37130	1995	IIb	Eiraku et al., 1995
HCM3	AF020629	1997	IIb	Fukushima et al., 1995d
ED		1996	IIa	Vandamme et al., 1998
ATL18_N0R2	U10258/U10252	1994	IIa	Switzer et al., 1995
FLN	L37134	1995	IIa	Eiraku et al., 1995
SAC	L37139	1995	IIa	Eiraku et al., 1995
SMH1_ASB	Y09147/L37143	1996	IIa	Eiraku et al., 1995
SMH2	Y09148	1996	IIa	Salemi et al., 1998
DOG	L37131	1995	IIa	Eiraku et al., 1995
Dub496	AF032993	1997	IIa	Egan et al., 1995
Dub991	AF032992	1997	IIa	Egan et al., 1995
Dub095	AF032991	1997	IIa	Egan et al., 1995
Dub408	AF032989	1997	IIa	Egan et al., 1995
Dub500	AF032990	1997	IIa	Egan et al., 1995
LA8A_MIN	U10256	1996	IIa	Switzer et al., 1995a
Iiend Subtype C				
KayCI	AF306733	2000	IIc	Ishak et al., 2001
KayCII	AF306732	2000	IIc	Ishak et al., 2001
KayCIII	AF306731	2000	IIc	Ishak et al., 2001
BH335	AY509602	2003	IIc	Ishak et al., 2001
BH315	AY509601	2003	IIc	Ishak et al., 2001
BH223	AY509600	2003	IIc	Ishak et al., 2001
New_END				
Gab	EU444100	2005	IIb	Lekana-Douki et al., 2008
GuyII	AF262408.1	2000	IIa	Lewis et al., 2000
Ty80	AF139391	1999	IIa	Lewis et al., 2000
Kay79	AF139389	1999	IIa	Lewis et al., 2000
Kay83	AF139390	1999	IIa	Lewis et al., 2000
NEW_IDU				
SP4	AY442367	1992	IIb	Toro et al., 2005
SP6	AY442369	1992	IIb	Toro et al., 2005
SP5	AY442368	1993	IIb	Toro et al., 2005
SP7	AY442370	1993	IIb	Toro et al., 2005
SP2	AY442365	1997	IIb	Toro et al., 2005
SP25	AY442388	2000	IIb	Toro et al., 2005
SP20	AY442383	2001	IIb	Toro et al., 2005

SP21	AY442384	2001	IIb	Toro et al., 2005
SP24	AY442387	2001	IIb	Toro et al., 2005
SP1	AY442364	2002	IIb	Toro et al., 2005
SP3	AY442366	2002	IIb	Toro et al., 2005
SP12	AY442375	2002	IIb	Toro et al., 2005
SP13	AY442376	2002	IIb	Toro et al., 2005
SP14	AY442377	2002	IIb	Toro et al., 2005
SP15	AY442378	2002	IIb	Toro et al., 2005
SP16	AY442379	2002	IIb	Toro et al., 2005
SP17	AY442380	2002	IIb	Toro et al., 2005
SP18	AY442381	2002	IIb	Toro et al., 2005
SP19	AY442382	2002	IIb	Toro et al., 2005
SP22	AY442385	2002	IIb	Toro et al., 2005
SP23	AY442386	2002	IIb	Toro et al., 2005
SP8	AY442371	2003	IIb	Toro et al., 2005
SP9	AY442372	2003	IIb	Toro et al., 2005
SP10	AY442373	2003	IIb	Toro et al., 2005
SP11	AY442374	2003	IIb	Toro et al., 2005
SP26	AY442389	2003	IIb	Toro et al., 2005

RESULTS

Many viruses, in particular RNA viruses, evolve very rapidly and thus satisfy the requirements to be a MEP. New statistical methods have recently been created to analyze the temporal information contained within MEPs. BEAST is one such method, which estimates substitution rates in a Bayesian MCMC coalescent framework given some calibration. Unlike Maximum Likelihood methods, Bayesian methods such as BEAST provide posterior estimates of evolutionary rates that are averaged across a large sample of genealogies, thus accounting for phylogenetic uncertainty (Duffy et al., 2008). Numerous other analyses have shown the significance of such methods, finding that rate determination can be commonly used to date evolutionary events, infer population dynamics and viral epidemics, as well as act as an independent, internal calibration for the age of species and populations (Korber et al., 2000; Pybus et al., 2001 and 2003; Lemey et al., 2003; Drummond et al., 2003).

Evolutionary Model

For each dataset, evolutionary models were chosen as described in the methods section. The results are found in Table 2.

Table 2. Evolutionary Model Selection for Individual Data Sets

Data Set	Evolutionary Model	Assumptions[†]
IIend	TrN (Tamura-Nei)	Variable base frequencies, equal transversion frequencies, variable transition frequencies
IIidu	TVM + G (Transversions Model)	Variable base frequencies, variable transversions, transitions equal
New_All_Sequences	K81uf + G (Kimura 3 Parameter; Unequal Frequencies)	Unequal base frequencies, equal transition frequencies, variable transversion frequencies

[†]Information taken from Bazinet, 2009.

Molecular Clock Rate

To determine whether the temporal information in the ages of the tips was useful in estimating the nucleotide substitution rate, we performed additional analyses on data sets IIend and IIidu using four different models: S_C, S_B, R_C and R_B (Nomenclature: First letter represents clock: Strict (S), Relaxed (R); and second letter represents coalescent prior: Constant (C) and Bayesian Skyline (B)). For both data sets, each model was run first incorporating the sampling age as temporal information, and then assuming that the tips were all sampled at the same time (isochronous) and thus, offering no temporal information. We used Bayes factors (Suchard et al., 2001) first, to determine which model using tip ages best fit our data and then to compare this model to the comparable isochronous model. The isochronous model was preferred over the tip-dated model, suggesting that the temporal data was not useful to inform the evolutionary rate for these HTLV-II data sets (Table 3). A separate analysis was performed with the alignment containing all sequences, New_All_Sequences. As with the other data sets, the model incorporating temporal information from the sequence ages was rejected in favor of the isochronous model. This test suggests that the rate of evolution in HTLV-II is too slow to calibrate the molecular clock analysis using the ages of the tips that are currently available.

Table 3. Results of Bayes Factors Comparison: Evolutionary and Isochronous Models

Phylogeny 1	Phylogeny 2	Log10 Bayes Factor
IIend_S_C	**IIend_S_C_Same**	.428
IIidu_S_C	**IIidu_S_C_Same**	.442
New_All_Sequences_S_C	**New_All_Sequences_S_C_Same**	.381

[†]Bolded datasets were selected for by bayes factors

[¥]Nomeclature is as follows: First letter distinguishes between Strict clock (S) and Relaxed clock (R); Second letter defines coalescent prior-Constant (C) or Bayesian skyline (B); third word defines whether clock was constricted to slow rate (Slow) or the entire range of rates identified for HTLV-II (fast); additional delta identifies a test where the delta parameter was implemented

Strict Rate Comparison

Next, we performed additional analyses to determine whether HTLV-II in IDUs evolves at a different rate than HTLV-II infecting ENDs. As described in the methods section, we performed two analyses in which the data set New_All_Sequences was constrained to two previously identified rates. Tests were undertaken to determine the most appropriate demographic model for this data set. Bayes factors indicated that a constant population size coalescent prior was a better fit than the flexible, Bayesian skyline coalescent prior (Drummond et al., 2005) for both the fast and the slow rate. In addition, Bayes factor comparison showed that the slower clock model was preferable to the faster clock model (Table 4).

Specific Delta Model

Lastly, we performed two additional tests incorporating a new parameter, the specific delta model. We first tested whether the fast or slow rate was a better fit to the data when the delta parameter was included by comparing the two models, S_C_Slow_Δ and S_C_Fast_Δ, As above, Bayes factors support the slower rate model, S_C_Slow_Δ (Table 3). Finally, to determine whether the addition of the delta parameter provides a better fit for the data then a model without the delta parameter, we compared .S_C_Fast_Δ-which assumed a strict clock, constant coalescent prior, a rate constricted to the full range identified for HTLV-II by previous authors (Salemi et al., 1998 and 1999; Lemey et al., 2005) and the delta parameter imposed on the IIidu sequences, with S_C_Slow. Bayes factor comparison shows an insignificant amount of support in favor of the Delta Model (Table 3).

Table 4. Results of Bayes Factor Comparison between Differing Evolutionary Models

Phylogeny 1	Phylogeny 2	Log10 Bayes Factor
S_C_Slow	S_C_Fast	4.722
S_C_Slow_Δ	S_C_Fast_Δ	5.303
S_C_Slow_Δ	**S_C_Slow**	.067

†Bolded datasets were selected for by bayes factors

DISCUSSION

The analyses presented in this study were helpful in investigating whether the HTLV-II virus has a different rate of evolution depending on mode of transmission (vertical vs. horizontal). Under the assumption that there was no difference in rate, analyses were undertaken using an updated set of global HTLV-II sequences and a flexible Bayesian MCMC statistical framework, resulting in the conclusion that no rate difference exists between these two mechanisms.

Molecular Clock Rate

In the current study, tests were performed to determine whether the data sets IIend and IIidu were evolving according to a molecular clock. For each data set, we assumed both a strict and relaxed molecular clock model. The ages of each sampled sequence within the data sets were used to calibrate each model. As the demographic histories of the viral populations were also unknown, it was necessary to perform a test to determine which model, a constant population size or a flexible, Bayesian Skyline (Bayesian

Skyline plot; Drummond et al., 2005) coalescent prior was more appropriate. In each case, these models were then compared to an isochronous model in which the tips were assumed to be sampled at the same time, thus offering no temporal information. Additional tests were performed on the dataset containing all sequences, New_All_Sequences. For each of the four models and the analyses on the dataset New_All_Sequences, Bayes factors supported the isochronous model. This result suggests that the rate of evolution in HTLV-II is too slow to calibrate a molecular clock using the ages of the sequences that are currently available in GenBank. This confirms an analysis performed by Lemey et al., 2005, who found that variation in HTLV-II fixes slower than most retroviruses. It also begs the question whether previous studies (Salemi et al., 1998 and 1999; Lemey et al., 2005)- which found, using differing methods, that HTLV-II in IDUs mutates at a rate comparable to most ssRNA viruses-are accurate. These results are best explained by HTLV-II's replication dynamics. Wattel et al. 1995 found that HTLV-II does not appear to exploit its capacity to generate diversity through its reverse transcriptase. Once transcribed the DNA is integrated into the host cell genome via an integrase and subsequently utilizes the host cells DNA polymerase for replication. As this polymerase makes use of a proof-reading exonuclease, it is unlikely after integration for sequence diversity to appear over a short period of time (Lemey et al., 2005). This would account for the lack of diversity necessary within the sampled time period for HTLV-II tip dating to calibrate our molecular clock.

Strict Rate Comparison

Additional analyses were undertaken to determine whether HTLV-II in IDUs mutates at a different rate than ENDs. As described in the methods section, assuming a strict clock model, we performed additional analyses assuming two previously identified rates that had been generated using internal calibrations in the literature. The first rate was representative of the full range of possible rates identified in previous studies for HTLV-II (Salemi et al., 1998 and 1999; Lemey et al., 2005). Conversely, the second rate was constrained to a range identified only as containing rates attributed to the slowly evolving sequences belong to endemically infected tribes (Salemi et al., 1999; Lemey et al., 2005). We then used Bayes factors to determine which rate was a better fit to the data. If the fast rate was selected as the best fit, this may give some credence that the IDU sequences evolve at a faster rate than the END sequences, and that this substitution rate skews the data towards an intermediary value only found within the full range of rates. If, on the other hand, the slow rate was selected, this may give credence to the assertion that HTLV-II evolves at a consistent slow rate. Our results show that the slower model, S_C_Slow, is a better fit than the faster model, S_C_Fast (Table 3). Although previous authors have claimed that viruses in IDUs evolve at a faster rate than ENDs (Salemi et al., 1998 and 1999; Lemey et al., 2005), this result offers evidence that regardless of transmission mechanism the substitution rate for HTLV-II may be the slow rate previously identified only for ENDs. However, because we imposed a strict clock model on the data, further work is necessary to confirm this result and the use of a strict clock model.

Specific Delta Model

To further investigate whether the IDU sequences were evolving at a different rate than the END sequences, we implemented a new parameter, the Specific Delta Model (SDM). Whereas a relaxed clock model imposed on the data would allow for a distribution of evolutionary rates across the tree, the SDM has both fewer parameters and provides an explicit test of whether a different rate of evolution exists within a specified subset of the data, in this case, the IDU sequences. Bayes factors were used to compare SDMs constricted to both the slow rate, identified only for ENDs and the full range of rates (the fast rate) identified for HTLV-II and selected for the slow rate, S_C_Slow_Δ (Table 3).

An additional test was undertaken, to compare the SDM to the single slow rate model to determine which was a better fit for our data. Because the SDM allows for the possibility that the IDU sequences, a subset previously identified as evolving at a faster rate than the END sequences (Salemi et al., 1998 and 1999; Lemey et al., 2005), evolve at a different rate, a Bayes factor comparison between the SDM and the single slow rate model would enable us to determine whether the IDU sequences evolve at a faster rate than the END sequences. If models including the SDM are a better fit than models without this parameter, this suggests that the sequences on which this parameter is imposed are more likely to be evolving at a faster rate than the sequences on which the parameter is not imposed. We found a log10 Bayes Factor of .067 in favor of the SDM, an insignificant result. In such a situation the simpler model, S_C_Slow, is chosen. This result supports our hypothesis that HTLV-II evolves at a constant rate, regardless of the mechanism of transmission. This result further supports the findings from the Strict Rate Comparison, that the constant rate at which HTLV-II evolves is that of the slower rate originally only associated with endemically infected tribes.

Conclusion

Our results support previous results suggesting that HTLV evolves more slowly than other RNA viruses. However, our results do not support the conclusions of Salemi, which suggest that HTLV-II evolves at different rates depending on the mode of transmission. The strongest support for this finding is in the rejection of the model with the delta parameter, which implies a common rate for the entire phylogeny.

Future research should focus on expanding the temporal depth of datasets in an effort to increase represented diversity. Such an increase may enable us to treat HTLV-II as a MEP, and thus use the statistical methods associated with such a condition. Additionally, the use of tip dating over internal calibrations, given such a scenario, would enable an independent confirmation for the yet, unsupported, Bering calibration.

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My research deals with the differences in evolutionary rates of HTLV-II in Intravenous drug users (horizontal transmission) as compared with infected indigenous tribes (vertical transmission).