

Selection against Pathogenic mtDNA Mutations in a Stem Cell Population Leads to the Loss of the 3243A→G Mutation in Blood

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The mutation 3243A→G is the most common heteroplasmic pathogenic mitochondrial DNA (mtDNA) mutation in humans, but it is not understood why the proportion of this mutation decreases in blood during life. Changing levels of mtDNA heteroplasmy are fundamentally related to the pathophysiology of the mitochondrial disease and correlate with clinical progression. To understand this process, we simulated the segregation of mtDNA in hematopoietic stem cells and leukocyte precursors. Our observations show that the percentage of mutant mtDNA in blood decreases exponentially over time. This is consistent with the existence of a selective process acting at the stem cell level and explains why the level of mutant mtDNA in blood is almost invariably lower than in nondividing (postmitotic) tissues such as skeletal muscle. By using this approach, we derived a formula from human data to correct for the change in heteroplasmy over time. A comparison of age-corrected blood heteroplasmy levels with skeletal muscle, an embryologically distinct postmitotic tissue, provides independent confirmation of the model. These findings indicate that selection against pathogenic mtDNA mutations occurs in a stem cell population.

Introduction

Individuals with pathogenic mutations in their mitochondrial DNA (mtDNA) typically have a mixture of wild-type and mutant mtDNA in each cell, a condition called heteroplasmy.¹ A heteroplasmic cell can compensate for the presence of mutant mtDNA until a certain threshold in the mutation level is reached, beyond which cell function is compromised.² The level of mtDNA heteroplasmy can vary across the tissues in an individual^{3–6} and can even vary across the cells within a single tissue.^{7–9} For the most common heteroplasmic pathogenic mutation, 3243A→G (MIM #590050.0001),^{10,11} the general pattern to the tissue variation is that the level of this pathogenic mtDNA mutation is lower in dividing cell types, and cells recently derived from dividing cells, than it is in long-lived postmitotic cells in the same individual. Specifically, mtDNA 3243A→G mutation levels in blood samples are almost always lower than the mutation level determined from a muscle biopsy.^{3,4} The tissue variation and the variation with time of mtDNA heteroplasmy are dependent on the particular mutation. Most of the available clinical data is for the 3243A→G mutation heteroplasmy, and we focus primarily on this mutation in this analysis. Longitudinal clinical studies have shown that 3243A→G blood heteroplasmy levels tend to slowly decrease over time.^{12–14} This is generally thought to occur through selection at the cellular level, but it is not clear whether this occurs in the stem cell population or the proliferating and differentiating blood cell precursors. The pathogenesis of diseases caused by mtDNA mutations is driven by the loss of function of a cell type or the actual loss of the cells themselves. The specific phenotype depends on the specific cell type

most strongly affected in that subject, due to intrinsic variability in the heteroplasmy levels across tissues, sensitivity of the cell (and the subject) to cofactors, or high energy requirements of the cell type. Understanding the long-time-scale dynamics of mtDNA heteroplasmy in different cell populations in the body is a basic step in understanding the pathology and the progression of these diseases.

In this paper, we analyze clinical data from two sets of patients: those with the 3243A→G mutation and those with the 8344A→G mutation (MIM #590060.0001). The 3243A→G mutation occurs in one of the mtDNA leucine transfer RNA (tRNA) genes (*MTTL1*). Although this mutation was first described¹⁵ in mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS [MIM 540000]), the spectrum of phenotypes caused by this mutation is extremely broad, extending from diabetes and deafness to hypertrophic cardiomyopathy and retinitis pigmentosa. This high phenotypic variability might in part be due to tissue-specific differences within each individual in the heteroplasmy level of the mutation. The 8344A→G mutation occurs in the mtDNA lysine tRNA gene (*MTTK*). This mutation is associated with Myoclonic Epilepsy with Ragged-Red Fibers Syndrome (MERRF [MIM #545000]) and can also cause multiple lipomata, cardiomyopathy, optic atrophy, myopathy, ataxia, and dementia. The 8344A→G mutation is also one of many mutations associated with Leigh Syndrome (MIM #256000), a relapsing encephalopathy that usually presents in childhood and is characterized by lesions in the basal ganglia.

Although the presence of a mtDNA mutation in blood rarely causes a clinical problem, understanding why heteroplasmy changes in blood has important implications.

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First, this tissue type can be affected by mtDNA mutations because large-scale deletions of mtDNA can lead to pancytopenia due to bone marrow aplasia (Pearson syndrome [MIM #557000]¹⁶). Second, the decreasing level of mutant mtDNA in blood presents a diagnostic challenge because the mutation might not be detectable in blood samples from affected individuals with standard techniques.¹⁷ Third, changing heteroplasmy levels with time introduces a major confounding variable when one is studying the transmission of mtDNA heteroplasmy, compromising studies of the mtDNA genetic bottleneck in humans¹⁸ and limiting the development of genetic-counseling guidelines. Finally, understanding the mechanisms behind changing heteroplasmy levels in blood is likely to have broader implications for other stem cell populations and the role of age-related diseases.¹⁹

A few serial measurements of 3243A→G heteroplasmy have been made in blood samples from the same patient at different time points. These studies reported a decrease in the proportion of mutant mtDNA by approximately 0.5% to 2% per year,¹² on the basis of pairs of measurements separated by several years. With this limited information, only a single value for the rate of decrease of mtDNA blood heteroplasmy can be calculated for each subject. It has not been known whether the variability in the measured rate of heteroplasmy decrease is due to variation across subjects or variation in the rate over time. However, on the basis of the simulation results reported here, we have reanalyzed published data to show that the rate of decrease in 3243A→G blood heteroplasmy is not constant, but is instead exponentially decreasing with time in a consistent manner across many subjects.

We have separated the problem of changes in blood heteroplasmy level into two questions. First, how does the heteroplasmy level in the hematopoietic stem cell population change over time? Second, how well does the heteroplasmy level in peripheral blood represent the heteroplasmy levels in the hematopoietic stem cell population? We have developed two separate simulations to address these two questions.

Material and Methods

Clinical Data

The clinical data were identified from a systematic search of the literature. Papers were identified that contained both the heteroplasmy level for an mtDNA point mutation measured in blood and the subject's age at sample collection. The focus was on identifying papers reporting data for multiple subjects. We concentrated on the 3243A→G and 8344A→G mutations because of the relatively large amount of data available for these two mutations. A total of 275 unique data pairs were identified for 3243A→G,^{3,4,12–14,20–37} and 48 unique data pairs were identified for 8344A→G.^{38–42} For the 3243A→G mutation, a subset of these studies was identified that also reported mtDNA heteroplasmy in muscle tissue.^{3,20–22,24,29,30,35,36} In cases where the data were only given in graphical form, the approximate numerical values

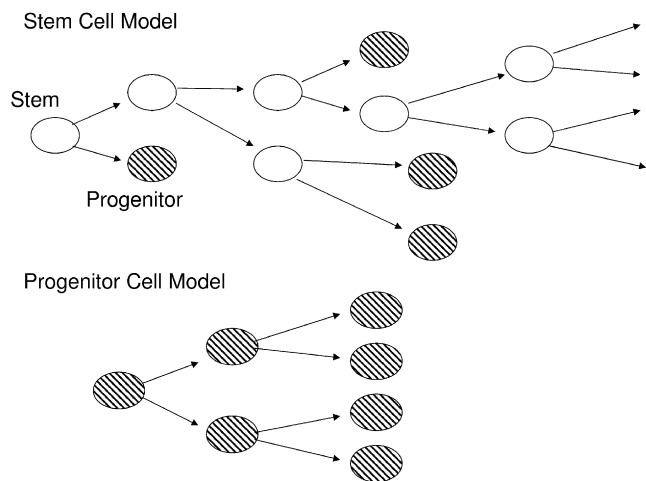


Figure 1. Schematic Diagram of the Two Cell-Division Models Open ovals represent stem cells. Shaded ovals represent progenitor cells.

were determined from these published graphs with the software Engauge Digitizer. For the analysis of changes in blood heteroplasmy levels over long time scales, one study was identified that had two measurements of 3243A→G in blood separated by 9 to 19 years, for six subjects.¹² To extend this dataset, our collaborators gathered this data for a further 11 subjects.¹⁴

Simulations

We used two related simulations: a stem cell model (Figure 1A) and a model of the rapid expansion of blood progenitor cells (Figure 1B). The simulation parameters are summarized in Table 1. In the stem cell model, we simulated populations of 20,000 to 2,000,000 stem cells with up to approximately 1000 to 2000 mtDNA molecules per cell for a maximum duration of 100 years. The actual number of mtDNA per hematopoietic stem cell is unknown, so we used a range of values and used the simulation results to determine the behavior for an arbitrary mtDNA copy number per cell. For simplicity, the initial heteroplasmy level in all stem cells was set to a uniform value, P_0 . In the progenitor cell model, we began with a single cell and modeled a series of approximately 20 cell divisions^{43,44} to form the mature blood cells.

Both simulation models contained many common features. Each mtDNA molecule in the simulation can be of either a wild-type mtDNA or a mutant-type mtDNA. We only considered point substitution mutations in this study because the dynamics of deletions might be different. Each simulated cell had integer values W and M representing the number of wild-type and mutant mtDNA in that cell. These numbers changed over time because of the processes of mtDNA replication (Figure 2A), degradation (Figure 2B), and cell division^{45,46} (Figure 2C). Note that although the total number of mtDNA molecules in the simulated cell is $N = W + M$, the total number N was not held constant over time and varied as W and M varied independently. The variables W and M were the basic variables of this model.

Cell Division

At each cell division, we assumed that the mtDNA molecules in the original cell were distributed randomly to each daughter cell with equal probability (Figure 2C). We modeled this with a Poisson distribution separately for the values W and M , with a mean value of $W/2$ and $M/2$ molecules distributed to each daughter cell. The sum

Table 1. Simulation Parameter Values

Parameter	Meaning	Values
D	Mean time between cell divisions	1 year (stem cell model) or 1 day (progenitor model)
T_{half}	mtDNA half-life	10 to 20 days
m_{thresh}	Cells with mutation level greater than m_{thresh} were removed from the simulation.	60% to 100%
N_t	Target number of mtDNA molecules in simulated cell (used to set replication rate). Also used to set the initial copy number in the cell.	1000 to 2000
P_0	Initial mutation level in cell	0% to 100%
Δt	Simulation time step	1 hr
No symbol	Total simulation duration	100 years (stem cell model) or 20 to 25 days (progenitor model)
No symbol	Total number of cells	20,000 to 2,000,000 (stem cell model) or 1 to 1,000,000 (progenitor model)

of the mtDNA copy numbers in the two daughter cells equaled the copy number in the original cell. The timing of the cell division was stochastic, with mean value D for the time between cell divisions. For the hematopoietic stem cell model, we set $D = 1$ year,⁴⁷ and for the blood progenitor model, we set $D = 1$ day.⁴⁴ In the stem cell model (Figure 1A), the two daughter cells were randomly assigned identities as stem cells or progenitor cells, with equal probability^{48,49} (because we were modeling the consequences of stem cell division, we did not incorporate the complicated feedback mechanisms controlling the stem cell self-renewal⁵⁰ because these detailed mechanisms were not considered relevant to this problem). The daughter cells labeled as “stem cells” were followed through subsequent cell divisions, and the daughter cells labeled “progenitor cells” were removed from this simulation, which was designed to only model the hematopoietic stem cell population. In the blood progenitor model (Figure 1B) all daughter cells were labeled as progenitor cells, and were retained in that simulation.

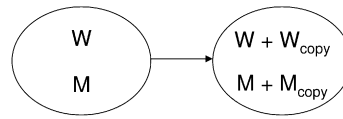
Degradation

Mitochondrial DNA molecules were removed from the simulation (Figure 2B) with a half-life, T_{half} , which we typically set in the range 10 to 20 days.⁵¹ Both wild-type and mutant mtDNA were degraded with the same half-life. In one time step of length Δt , the number of wild-type mtDNA molecules lost to random degradation (W_{loss}) was calculated from a Poisson distribution with mean value of $(\ln(2) W \Delta t / T_{half})$, where W was the number of wild-type mtDNA in the cell. A similar calculation determined the number of mutant mtDNA molecules degraded.

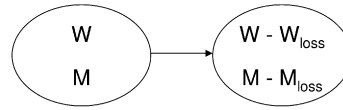
Mitochondrial DNA Replication

The simulated cells copied their mtDNA (Figure 2A) at a rate R to compensate for the loss of mtDNA by degradation and to repopulate the cell with mtDNA after cell divisions. Let N_t be the target total number of mtDNA in the cell. Note that N_t was merely a parameter used for the calculation of the mtDNA replication rate and that it might be slightly different than the total number of mtDNA molecules actually present in the simulation at a given time, $N = W + M$. In steady state, the actual copy number N fluctuated

A Relaxed Replication



B Random Degradation



C Random Segregation

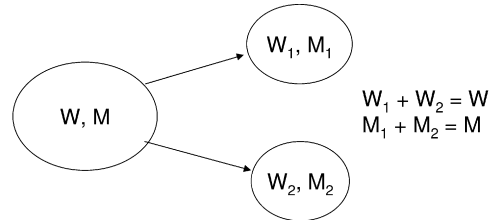


Figure 2. Schematic Diagrams of the mtDNA Dynamics in the Models

(A) In relaxed replication, the number of wild-type (W) and mutant (M) mtDNA molecules was increased over one time step by the amounts W_{copy} and M_{copy} , respectively.

(B) In the random-degradation model, the numbers W and M were decreased over one time step by W_{loss} and M_{loss} .

(C) At a cell-division event, the values for W and M were randomly distributed to the two daughter cells.

about the target number N_t . The mtDNA replication rate R was modeled as a sum of two terms:

$$R = (\ln(2)N_t/T_{half}) + (\ln(2)N_t/D). \quad (1)$$

The first term was the replication needed to replace degraded mtDNA. The second term was the replication rate needed to compensate for the decrease in mtDNA copy number from cell divisions. This replication rate was distributed proportionally over the mutant and wild-type mtDNA in the cell. The total number of wild-type mtDNA copied (W_{copy}) and mutant mtDNA copied (M_{copy}) in one time step of length Δt was $R \Delta t = W_{copy} + M_{copy}$ (Figure 2A). The separation into W_{copy} and M_{copy} was made through a binomial probability distribution.

Cell Loss

For the modeling of the pathogenic effect of the mutant mtDNA, any cell with a mutation level above a set threshold m_{thresh} was removed from the simulation. Values in the range $60\% < m_{thresh} < 100\%$ were used in the simulation. This range covers the range of reported values for the threshold of 3243A \rightarrow G.^{52,53} This model of cell loss was used in both the hematopoietic stem cell simulation and in the blood precursor simulation.

Measurements of the Rate of Heteroplasmy Decrease

To remove the effect of an initial transient (the shoulder in the initial short period of simulation in Figure 3A) on the measurement of the heteroplasmy decay rate, we only used simulation data after the mutation level had dropped to below 90% of its initial value. Because the average mutation level in the simulation decreased

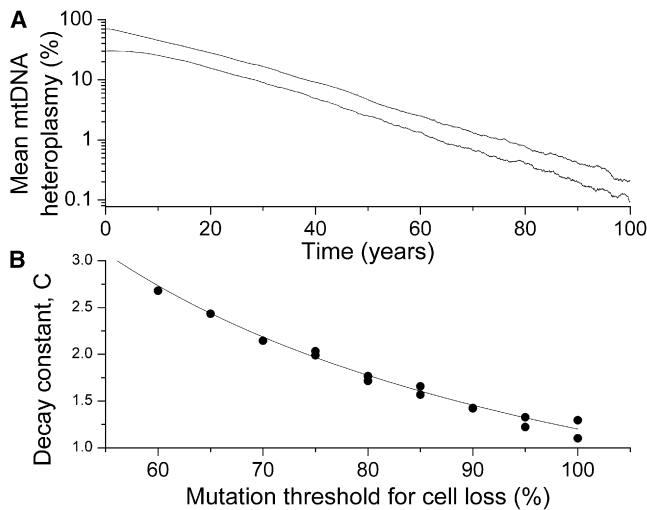


Figure 3. Simulation Results for the Blood Stem Cell Model
 (A) An exponential decrease in mean mutation level in blood stem cell model. Results are shown for two simulations with different initial mutation levels, 70% and 30%. Other simulation parameters were $T_{\text{half}} = 10$ days, $N = 1000$, and $m_{\text{thresh}} = 90\%$.
 (B) Dependence of the decay constant C , defined in Equation 2, on the mutation threshold for loss of the stem cell, m_{thresh} . The line is a fit to the data with Equation 3 (with $R^2 = 0.968$ and $p < 0.0001$).

over time, we only used the mutation-level values down to 10% mutant, to remove the effects of noise due to discreteness at low levels of mutant mtDNA copy number per cell.

Results

Hematopoietic Stem Cell Simulation Results

The processes of relaxed replication of mtDNA,⁵⁴ degradation of mtDNA, and random segregation between daughter cells all caused the heteroplasmy levels in this population of stem cells to spread out. As the distribution of heteroplasmy widened through random genetic drift,^{55,56} some cells developed a high mutation heteroplasmy level and were removed from the simulation, modeling the pathogenic effect of the mtDNA mutation. Over time, this loss of cells with high mtDNA mutation level gradually decreased the average mutation level of the simulated hematopoietic stem cell population. Decreasing heteroplasmy levels in blood have been reported for the 3243A→G mutation,^{12–14} based on two measurements spaced over a few years. These measurements are usually interpreted as a simple linear rate of decrease in the mutant mtDNA levels in blood samples. Our simulation results suggest that the rate of loss of heteroplasmy from blood stem cells is actually exponential (Figure 3A), not a constant linear rate.

In the model, the average mutation level as a function of time was described by a decaying exponential, after an initial transient period (Figure 3A). By varying the model parameters [mtDNA half-life T_{half} and initial copy number per cell $N_t = W(0) + M(0)$], we found that the average mu-

Table 2. The Value of the Exponential-Decay Constant C in Equation 2 Computed for Various Simulation Parameters

Number of Stem Cells	mtDNA Copy Number, N_t	mtDNA Half-Life, T_{half} (Days)	Initial Mutation Level, m (%)	C
20,000	1,000	10	30	1.4251
20,000	1,000	10	70	1.4212
20,000	1,000	20	30	1.5234
20,000	1,000	20	70	1.5459
20,000	2,000	10	30	1.3870
20,000	2,000	10	70	1.3662
20,000	2,000	20	30	1.5530
20,000	2,000	20	70	1.4423
2,000,000	1,000	10	30	1.4075

The parameters N_t , T_{half} , and the initial heteroplasmy levels were varied. The mean decay constant in these simulations was $C = 1.45$, with standard deviation = 0.07.

tation level $m(t)$ as a function of time, t , had the functional form

$$m(t) = m_0 \exp\left[\frac{-C}{T_{\text{half}} N_t} t\right], \quad (2)$$

where m_0 was a constant approximately equal to the initial mutation level and C was a dimensionless constant, with the value of $C = 1.45 \pm 0.07$ determined from the simulations (Table 2).

Sets of simulations where the mutation-level threshold for cell removal, m_{thresh} , was varied showed that the decay constant C is a function of the mutation threshold (Figure 3B), with lower thresholds causing faster loss of the mutation from the stem cell population, an intuitively reasonable behavior. In the range $60\% \leq m_{\text{thresh}} \leq 100\%$, this dependence was well described by function

$$C = C_0 - (A/m_{\text{thresh}}), \quad (3)$$

where the constant values determined by a linear fit were $C_0 = -1.1 \pm 0.1$ and $A = (230 \pm 8)\%$. Even at a threshold of 100% (Figure 3B), requiring the simulated cell to fix on the mutation before the cell is removed, the simulation still showed an exponential decrease in heteroplasmy level in the hematopoietic stem cell population.

Blood Precursor Simulation Results

The hematopoietic stem cell simulation modeled the changes in the population of blood stem cells over time. To relate this to measurements taken from samples of peripheral blood, we needed to know how mtDNA heteroplasmy levels can change through the process of forming the mature blood cells from the stem cell population. We modeled this very simply as a series of rapid cell divisions with a mean cell division period of 1 day.⁴⁴ For the purpose of this model, we ignored all of the complications of the different types of blood cells, and we focused just on the basic question of how a rapid expansion in the cell population could affect the mean mtDNA heteroplasmy level

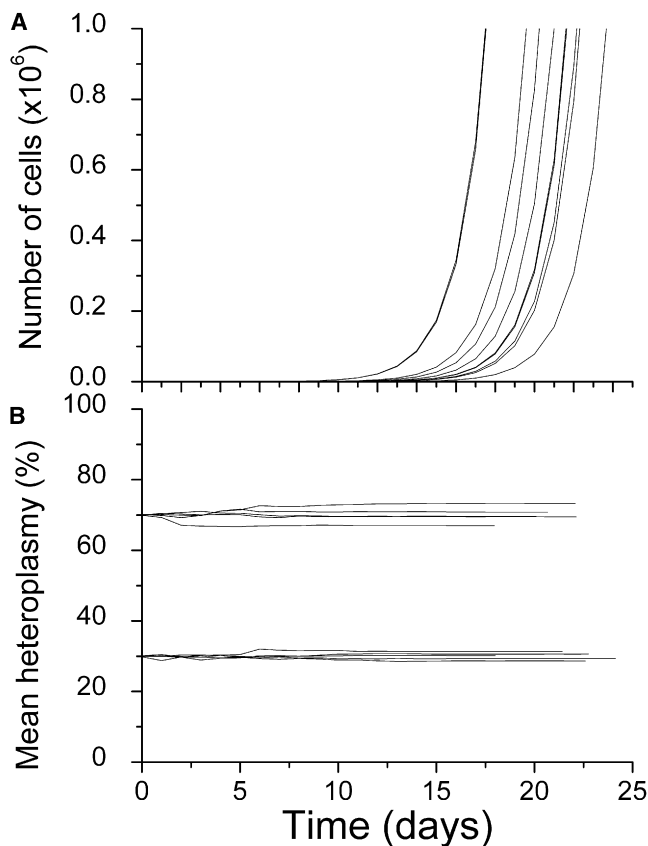


Figure 4. Simulation Results for the Progenitor Cell Model
 Results are shown for five repetitions of the simulation starting at 70% mutant and five repetitions starting at 30% mutant.
 (A) The exponential increase in the number of simulated cells, starting from a single progenitor cell and dividing on average once per day.
 (B) The mean heteroplasmy of the cells over the course of the expansion in the number of cells.

in these cells. Figure 4 shows a set of ten typical simulation results. Figure 4A shows the increase in the cell number as a function of time. The variation in this plot across repeated simulations shows the sensitivity of the total cell number to the stochastic timing of the first few cell divisions. This variation is not critical for the simulation results on mtDNA heteroplasmy levels. The primary result is in Figure 4B, where the mean mutant mtDNA level, $m(t)$, is shown. Throughout this expansion from a single initial cell, the mean mutant mtDNA level remained relatively constant, varying only a few percent. In this model of an exponentially expanding population of cells, there was no consistent shift in heteroplasmy level, as there was in the stem cell model, even though both simulations included the threshold mechanism for removing cells with high mutation heteroplasmy. Our conclusion is that the mean mtDNA mutation level measured in peripheral blood is a good measure of the mean mutation level in the stem cell population from which those cells were derived. Moreover, the lack of any significant effect of selection in the differentiating (non-stem cell) blood precursors

(Figure 4B) indicates that the selective mechanism must be acting at the stem cell level and not as the committed blood cell precursors mature. This is consistent with population genetic models and observations, which show that selection does not act significantly on an exponentially expanding population.⁵⁷

Analysis of Experimental Data

The primary results of our modeling were qualitative ones: first, that the mean mtDNA mutation levels in hematopoietic stem cells should decrease exponentially over long time scales, and second, that measurements of mutation levels in peripheral blood samples should be a good proxy for the mutation level in the stem cell population. To apply these concepts to the published clinical data on mtDNA mutation levels in blood, we first needed a way to determine whether the observed mtDNA heteroplasmy decreases exponentially, as predicted by the simulations. The existing clinical data consists only of pairs of measurements spaced over a few years at most. With just pairs of measurements, not a long time series, we can only calculate the rate of change of heteroplasmy. For an exponential decay, like Equation 2, the rate of change of heteroplasmy is not constant, but is instead proportional to the heteroplasmy level. Therefore, a plot of the rate of mtDNA heteroplasmy change versus initial heteroplasmy level should be a linear plot with zero intercept and negative slope. If the decay is constant instead of exponential, then data on this plot will lie in a horizontal line with zero slope.

Because the decrease of blood mtDNA heteroplasmy in the simulation was very slow (Figure 3A), we need clinical data with measurements separated in time by approximately a decade. The best existing clinical data available was from a study by Rahman et al.¹² where measurements of 3243A→G heteroplasmy in blood were separated by 9 to 19 years. However, that study only reported values for six individuals. To extend the dataset, we and our collaborators carried out repeat measurements of blood heteroplasmy on 11 individuals (all with the 3243A→G mutation) to compare to measurements that had been made 5 or more years previously. Those data have been published separately.¹⁴ The new data from the Pyle et al. study¹⁴ fit together well with the data from the Rahman et al. study¹² (Figure 5, note that this is plotted so that an exponential decay in mtDNA heteroplasmy with time shows as a straight line). Taken together, these two clinical datasets give a consistent picture of an exponential decrease in blood mtDNA heteroplasmy levels over time in a consistent manner in different individuals from different studies.

Serial measurements from the same patient are rare, particularly over a long time period. The vast majority of the available clinical data consist of heteroplasmy measurements at a single time or at relatively closely spaced times. Even those data can be used to test the predictions of this model. One intuitively obvious consequence of this exponential decay model is that we should not observe high 3243A→G blood heteroplasmy levels in older individuals.

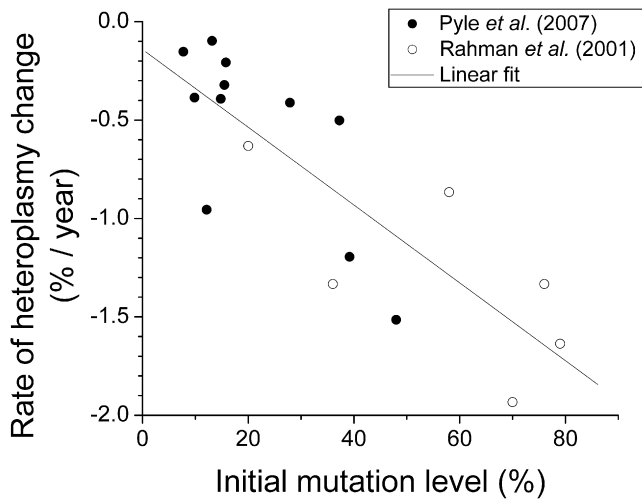


Figure 5. Analysis of Clinical Data to Test for an Exponential Decrease in Peripheral Blood Heteroplasmy

The data are taken from two independent experiments^{12,14} on the 3243A → G mutation. The line is a linear fit to the data, with slope -0.020 ± 0.003 (1/year) and intercept -0.14 ± 0.14 (%/year) ($R^2 = 0.68$ and $p < 10^{-4}$). A linear plot of this data with negative slope indicates an exponential decay of the blood heteroplasmy.

We can quantify this limitation by defining a maximum blood heteroplasmy level as

$$\max[m(t)] = (100\%)\exp(-St), \quad (4)$$

where $S = 0.020 \pm 0.003$ (1/years) was the slope measured from the data in Figure 5. Blood heteroplasmy measurements for 3243A → G mtDNA mutations should fall under this maximum limit. We identified 23 published studies^{3,4,12-14,20-37} reporting 3243A → G blood mtDNA heteroplasmy levels together with the subjects' age, with a total of 275 unique data points. The data lie below the predicted maximum heteroplasmy (Figure 6A) except for five measurements that lie just above the line, and the data extend from zero mutation up to the predicted maximum. There is a region of the plot, at young age and low heteroplasmy, with very few data points. This is likely to be just an ascertainment effect, because most patients with 3243A → G present with symptoms in their teenage years or older.

The few data points in Figure 6A that lie just above the predicted maximum heteroplasmy are not a concern. These could easily be caused by very slight individual variations in the rate of decrease of the 3243A → G mutation. Only cases that lie far above the predicted maximum heteroplasmy limit would be of concern. Four such cases were reported in Hammans et al.,²² and they were recognized by those authors as being very unusual. All four cases came from a single small pedigree that also contained a homoplasmic T to C transition at position 3290, in the same tRNA gene as the pathogenic 3243A → G mutation. The 3290T → C sequence variant was not found in 140 controls or 50 patients. Hammans et al. noted that this family had a unique clinical phenotype and suggested that this

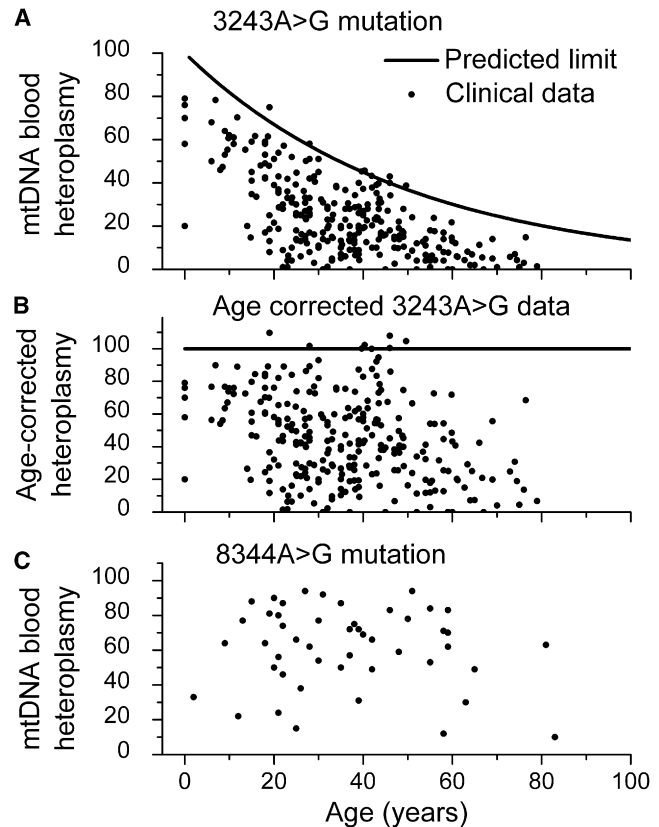


Figure 6. Comparison of Clinical Data to a Predicted Maximum Mutation Heteroplasmy in Peripheral Blood

(A) The theoretical maximum blood mutation level (Equation 4) and 275 measured values from 23 separate studies^{3,4,12-14,20-37} on subjects with the 3243A → G mutation. The theoretical upper limit is set with an initial heteroplasmy of 100%.

(B) The 3243A → G data modified by the age correction (Equation 5). (C) Blood heteroplasmy data (48 measured values) for the 8344A → G mutation.³⁸⁻⁴² The clinical results plotted here show no indication of a decrease in blood heteroplasmy with the subjects' age for this mutation.

3290T → C sequence variant might have altered the pathology of the 3243A → G mutation. El Meziane et al.⁵⁸ have also shown experimentally that a second mutation, at position 12300 in that case, can suppress the pathogenicity of the 3243A → G mutation.

On the basis of this analysis of the published data, we can suggest a simple method for correcting for the subject's age in blood heteroplasmy measurements. We defined an age-corrected mutation level as follows:

$$m_{\text{age-corrected}} = m(t)\exp(St). \quad (5)$$

These age-corrected blood heteroplasmy measurements are plotted in Figure 6B.

For the 3243A → G mutation, the heteroplasmy levels clearly decrease with age, and we have shown that the loss of hematopoietic stem cells with high heteroplasmy levels can lead to this exponential decrease. However, it is possible that for some other pathogenic mtDNA mutations,

these stem cells could remain viable even when homoplasmic for the mutation. In these cases, or in the case of neutral mutations, we would not expect to see a loss of the mtDNA mutation over time from the blood. These low-penetrance pathogenic mutations were excluded from the analysis of Figures 6A and 6B. One such mutation for which a significant amount of heteroplasmy data exists is the 8344A→G mutation. Indeed, when blood heteroplasmy measurements for subjects with this mutation^{38–42} were plotted as a function of age, an age-dependent upper limit to heteroplasmy did not appear (Figure 6C), indicating that this mutation is not significantly decreased in the blood over time.

In the 3243A→G mutation, measurements of blood heteroplasmy levels are almost always less than the heteroplasmy levels measured in muscle biopsies.³ For data sets where we have the measured heteroplasmy in blood and in muscle, along with the subjects' age, we can test to see whether the age-corrected blood heteroplasmy is consistent with the muscle heteroplasmy.^{3,20–22,24,29,30,35,36} In Figure 7A, we plot blood heteroplasmy versus muscle heteroplasmy for the 3243A→G mutation. The line marks equal heteroplasmy values. Although there is a significant correlation between the blood and muscle heteroplasmy values, all of the subjects had lower heteroplasmy in blood compared to muscle. Also, there is a pronounced age effect visible in this data, with older subjects generally having a greater difference between muscle and blood heteroplasmy. Assuming that muscle heteroplasmy is relatively constant over time, we applied the age correction of Equation 5 to the blood heteroplasmy levels (Figure 7B). Both the blood measurements and the age-corrected blood measurements are highly significantly correlated with the muscle heteroplasmy measurements ($p < 0.0001$ in both cases). The improvement is that the age-corrected data clustered around the line of equal heteroplasmy, though there was still some tendency for the age-corrected blood heteroplasmy to be lower than the muscle heteroplasmy, particularly for muscle heteroplasmy levels above 75%. However, the age stratification disappeared in the age-corrected data, with all three age groups overlapping.

Discussion

Our analysis of the clinical data relating mtDNA heteroplasmy levels of the 3243A→G mutation in blood to the subjects' age (Figures 5 and 6) indicates that the mutant heteroplasmy level does decrease exponentially with time, as predicted by the simulation. Our simulations of hematopoietic stem cells showed that the mechanism of loss of stem cells with high mutation level was sufficient to cause an exponential decrease in blood heteroplasmy. However, that does not mean that other mechanisms could not also lead to an exponential decrease. For example, Battersby et al.⁵⁹ have reported a very fast exponential decrease in blood heteroplasmy in mice constructed to

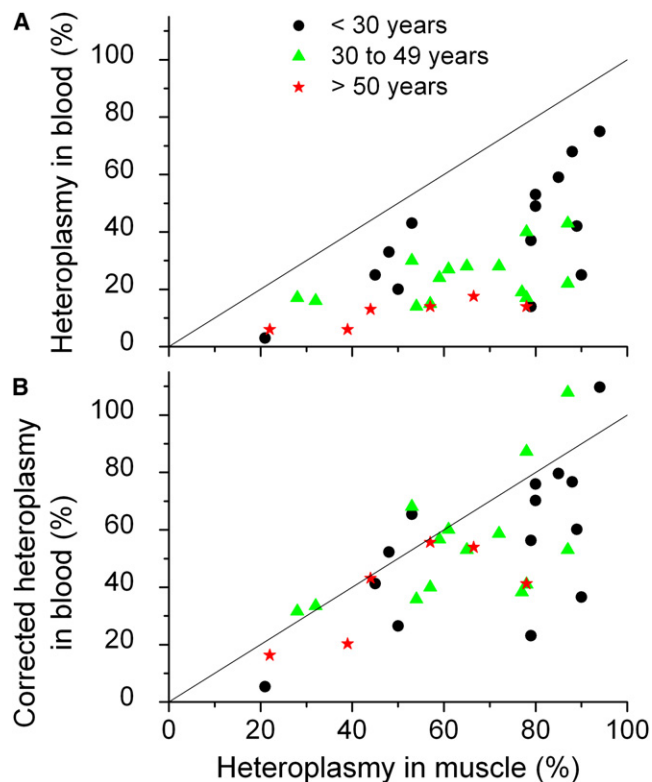


Figure 7. An Application of the Age Correction for Blood Heteroplasmy

(A) Comparison of 3243A→G heteroplasmy levels in muscle and in blood.

(B) Comparison with the blood heteroplasmy corrected for age (Equation 5). The corrections were made with a value of $S = 0.020 \text{ year}^{-1}$, the value determined from the data in Figure 5. The solid lines are the lines of equal blood and muscle heteroplasmy.

have a mixture of two naturally occurring nonpathogenic murine mtDNA strains, BALB/c and NZB. In these mice, the NZB mtDNA strain was lost from the blood with a decay constant $S_{\text{mouse}} = 1.387 \text{ year}^{-1}$ (almost 70 times faster than the value $S_{\text{human}} = 0.02 \text{ year}^{-1}$ from Figure 5). These authors test, and reject, the hypothesis that this selection against NZB mtDNA is occurring through an immune mechanism. They also did not find any functional difference in oxidative phosphorylation as a function of the cellular heteroplasmy level, indicating that a threshold effect and the cell-loss mechanism modeled in our simulation are likely not playing a role in those mice. The mechanism driving the rapid loss of NZB heteroplasmy in the blood of those mice is still unknown.

The data presented in Figures 5 and 6A, illustrating the exponential loss of pathogenic mtDNA from human blood, came exclusively from measurements on the 3243A→G mutation, the most commonly studied and the most commonly occurring¹⁰ heteroplasmic pathogenic mtDNA point mutation. Determination of whether this exponential loss occurs for other pathogenic mtDNA mutants will require either a number of pairs of longitudinal blood

heteroplasmy measurements spaced by approximately 10 years (Figure 5) or a much larger number of single-time-point measurements (Figure 6). In the latter case, it is important that a wide range of subject ages are sampled, so that the upper limit on blood heteroplasmy as a function of age can be detected.

We have focused our analysis on the 3243A→G mtDNA point mutations. However, this does not mean that other mtDNA mutations causing low-penetrance diseases, such as Leber's Hereditary Optic Neuropathy (LHON [MIM #535000]), cannot also show a decrease in blood heteroplasmy in some individuals. For those individuals who have the currently unknown cofactors that lead to the disease state, it is possible that the loss of high-heteroplasmy blood stem cells will occur (if this stem cell population is affected by the cofactors). Indeed, sporadic cases of decreasing blood heteroplasmy of LHON mutations have been reported.^{60,61} We can speculate that the observance of a decrease in blood heteroplasmy over time, or of a lower heteroplasmy in blood compared to muscle, in carriers of LHON mtDNA mutations could indicate the presence of the pathogenic cofactors in those individuals, even in cases where the overall heteroplasmy level of the LHON mutation might not be high enough to cause the disease state (along with the presence of the cofactor). In LHON, an interacting nuclear genetic locus⁶² or transient exposure to environmental toxins (such as excess alcohol or tobacco, see⁶³ for a positive correlation and⁶⁴ for no correlation) might act as cofactors for the development of the disease state.

We have also focused our analysis on blood samples, for obvious practical reasons. The same behavior of exponentially decreasing mtDNA heteroplasmy would also occur in any other tissue that would experience the loss of cells with high heteroplasmy. Decreasing 3243A→G mutation levels with age have been reported in epithelial cells^{4,65} and in buccal mucosa.⁴ In contrast, skeletal muscle is a large multinucleate postmitotic cell, and there is minimal muscle cell loss in patients even with high levels of 3243A→G.⁶⁶ This fundamental difference probably explains why mutant genomes tend to accumulate in nondividing tissues such as skeletal muscle,^{67,68} as we have discussed before.⁴⁶ Despite the known variability of blood heteroplasmy with age, blood samples are still very commonly used for the determination of heteroplasmy levels in individuals, most commonly in asymptomatic individuals. The use of blood samples for the determination of heteroplasmy might lead to misleading artifacts, such as the reported increase in heteroplasmy of a pathogenic mtDNA mutation across generations,¹⁸ a measurement that naturally entails comparisons of individuals with radically different ages. The possibility of this artifact has been raised before,⁶¹ however, this is the first time that a viable method of correcting for this artifact has been suggested.

Do the observations in blood have a broader relevance? Recent observation in colonic^{19,69} and gastric⁷⁰ tissue show that pathogenic point mutations of mtDNA accumu-

late with age within the stem cell niche of healthy subjects, causing mitochondrial dysfunction in the daughter cell population. The work described here is directly applicable to situations such as this if the mutation leads to cellular dysfunction or cell death in any self-renewing population. For hematological stem cells, at least, our observations do suggest that stem cell viability is partly dependent upon an intact mitochondrial respiratory chain. Our observations also provide some hope for developing autologous stem cell therapies for mtDNA diseases, where, paradoxically, disease progression is associated with the cleansing of the inherited mtDNA mutation from stem cells (an effect that is explained by the results of this study). These could be harnessed for the delivery of wild-type mtDNA to diseased cells, as has been demonstrated in human skeletal muscle.⁷¹⁻⁷³

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Web Resources

The URLs for data presented herein are as follows:

Engauge Digitizer, <http://digitizer.sourceforge.net>

Hematopoietic Stem Cell simulation codes, http://staff.vbi.vt.edu/dsamuels/2008_Blood_Stem_Cell_Paper/software/

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

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