

Barcode, Passenger, or Driver? Mitochondrial Heteroplasmy in the Somatic Evolution of Human Haematopoiesis

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Abstract

Human haematopoiesis is increasingly understood as a lifelong evolutionary process in which somatic variation, clonal competition and changing cellular environments reshape the contribution of individual haematopoietic stem cells. Most models of this process remain centred on the nuclear genome. Mitochondrial DNA introduces a second, genetically distinct substrate of somatic variation whose evolutionary role is less settled. Naturally occurring mitochondrial DNA variants can provide lineage information because they are abundant, clonally inherited and recoverable alongside single cell transcriptional or chromatin states. Large population genetic studies further suggest that many age associated mitochondrial heteroplasmies in blood behave predominantly as neutral passengers exposed by haematopoietic clonal expansion. Yet neutrality is not universal. Pathogenic mitochondrial variants undergo lineage dependent purifying selection, heteroplasmy associates with high risk clonal haematopoiesis and myeloid neoplasia, nuclear genetic variation regulates mitochondrial copy number and heteroplasmy, and *DNMT3A* R882 mutant haematopoietic stem and progenitor cells show genotype specific dependence on mitochondrial metabolism. This Review proposes a framework distinguishing mitochondrial variants acting as barcodes, passengers, fitness modifiers or, under a deliberately stringent evidentiary threshold, drivers. A fifth possibility, mitonuclear epistasis, considers clonal fitness as an emergent property of nuclear genotype, mitochondrial genotype, heteroplasmy, lineage and cellular context. Distinguishing these states will require longitudinal, lineage resolved and experimentally perturbable models. Mitochondrial heteroplasmy may therefore be more than an instrument for observing somatic evolution: in defined contexts, it may form part of the evolutionary system itself.

Introduction: two genomes within one evolving clone

Human blood is not genetically static. Throughout life, haematopoietic stem and progenitor cells acquire somatic mutations, compete for representation within a finite stem cell compartment and transmit successful genotypes to differentiated progeny. Ageing progressively alters this population structure. Some clones remain small for decades, others expand slowly, and a minority achieve sufficient competitive advantage to dominate a substantial fraction of haematopoiesis or progress towards myeloid neoplasia. The discovery of age related clonal haematopoiesis established the biological and clinical importance of this somatic mosaicism, while subsequent population modelling and longitudinal sequencing demonstrated that mutation, drift and gene specific fitness effects jointly shape clonal trajectories [1–4].

The dominant evolutionary framework for this process is nuclear. Mutations in *DNMT3A*, *TET2*, *ASXL1*, *JAK2*, *TP53* and spliceosome genes are interpreted as heritable cellular variants upon which selection acts. Mutation acquisition, clone size, competing clones, age and cellular environment then influence persistence or expansion. This framework has transformed clonal haematopoiesis from a descriptive molecular observation into a tractable problem in somatic population genetics. Every haematopoietic cell, however, contains another genome. The mitochondrial genome is small, multicopy and genetically unusual. Multiple mitochondrial genotypes can coexist within an individual cell, producing heteroplasmy,

and the relative abundance of those genotypes can vary between cells, lineages, tissues and time. Human population data further demonstrate that mitochondrial copy number and heteroplasmy are themselves influenced by nuclear genetic variation, underscoring the biological interdependence of the two genomes [5–7].

This creates an unusual evolutionary problem. A mitochondrial DNA variant detected in blood may simply record the ancestry of an expanding nuclear clone without contributing to that expansion. It may instead be a neutral passenger whose apparent enrichment reflects proliferation of the cell in which it happened to reside. Alternatively, mitochondrial genotype may alter cellular physiology sufficiently to influence survival, differentiation or self renewal. Most intriguingly, its effect may depend on the nuclear genotype with which it coexists. The central question is therefore not whether mitochondrial heteroplasmy occurs in haematopoiesis, but what evolutionary role a particular mitochondrial variant plays in the cellular clone that carries it.

This Review develops a framework in which mitochondrial heteroplasmy can be interpreted as a barcode, a passenger, a fitness modifier or, under a much higher evidentiary threshold, a driver. It further proposes that the most biologically informative unit may sometimes be neither genome considered independently, but a mitonuclear genotype whose fitness emerges from interaction among nuclear mutation, mitochondrial variation, heteroplasmy, lineage and cellular state.

Scope and approach of this Review

This is a critical narrative Review rather than a systematic review. The synthesis prioritises peer reviewed literature from approximately the past decade, with earlier seminal studies included where necessary to establish clonal haematopoiesis or fundamental mitochondrial genetic principles. Particular emphasis is placed on recent human population genomics, single cell lineage tracing, mitochondrial heteroplasmy, lineage specific selection, clonal haematopoiesis and experimentally demonstrated mitochondrial dependencies. Contradictory evidence and methodological limitations are considered explicitly because the central objective is not to determine whether mitochondrial heteroplasmy is uniformly neutral or functional, but to define the evidence needed to distinguish those states in human haematopoiesis.

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Mitochondrial heteroplasmy is not equivalent to an ordinary somatic mutation

The mitochondrial genome differs from the nuclear genome in ways that fundamentally alter evolutionary interpretation. Human mitochondria contain multiple copies of a compact circular genome, and cells can harbour tens to thousands of mtDNA molecules depending on cellular identity and

physiological state. Consequently, a cell does not simply carry or lack a mitochondrial variant; the relevant variable is often the fraction of mtDNA molecules carrying that variant. This heteroplasmy level can differ markedly between daughter cells because mitochondrial genomes undergo replication, turnover and segregation independently of the precise chromosomal partitioning that characterises nuclear inheritance [5–7].

Mitochondrial variants are therefore subject to nested evolutionary processes. Replicative competition can occur among mtDNA molecules within the same cell, while selection can simultaneously act among cells carrying different mitochondrial states. Heteroplasmy can also display threshold dependent functional effects: a low mutant fraction may remain phenotypically silent whereas higher levels can compromise respiratory function or produce other cellular consequences. The threshold is not universal; it varies according to the mutation, tissue and metabolic demands of the cell [5, 8].

Purifying selection further complicates interpretation. Population constraint analyses demonstrate marked depletion of deleterious mitochondrial variation, indicating that the mitochondrial genome is under substantial selection despite its unusual inheritance [9]. Within human haematopoiesis, pathogenic variants can also be depleted selectively from particular cellular compartments. Studies of mitochondrial disease have demonstrated especially strong negative selection during T cell differentiation, showing that one mitochondrial genotype can experience different selection pressures in different blood lineages [10–12].

A measured increase in heteroplasmy can therefore arise through expansion of mutant mtDNA molecules within cells, expansion of cells containing the variant, or both. Conversely, decreasing heteroplasmy can represent intracellular purifying selection, preferential loss of high heteroplasmy cells, lineage dependent differentiation or replacement by another cellular clone. The biological level at which selection acts must therefore be identified before selection itself is inferred.

The barcode state: when mtDNA records ancestry

The clearest established role for somatic mtDNA variation in human haematopoiesis is as a source of natural lineage information. The mitochondrial genome combines high copy number with comparatively frequent sequence variation, creating naturally occurring differences that can distinguish cellular lineages without engineered genetic labels. Ludwig et al. [13] showed that somatic mtDNA variants could be detected through single cell genomic assays and used to infer cellular relationships while simultaneously retrieving information about gene expression or chromatin state. Lareau et al. [14] subsequently developed massively parallel mitochondrial genotyping with chromatin profiling, expanding the scale at which mitochondrial variation could be integrated with cellular phenotype.

This approach was advanced further by Weng et al. [15], whose ReDeeM strategy combined deep mitochondrial mutation profiling with transcriptomic and chromatin accessibility measurements. Applied to human haematopoiesis, the method reconstructed putative clonal relationships and suggested stable heterogeneity in the output and lineage preferences of individual HSC clones, together with decreasing

clonal diversity with age. These observations illustrated the potential power of mtDNA as an endogenous record of haematopoietic history.

The assumption of a straightforward natural barcode, however, now requires greater qualification. Wang et al. [16] used computational modelling and single cell data to show that the performance of mitochondrial lineage tracing depends strongly on the degree of clonal expansion. Many apparently lineage specific mtDNA variants can pre exist as heteroplasmies rather than arise de novo during the lineage being reconstructed, whereas reliable discrimination improves in settings of strong cellular expansion. More recently, Lareau et al. [17] challenged aspects of the ReDeeM analysis, arguing that low support and edge associated mtDNA calls could introduce artefactual phylogenetic structure. Weng et al. [18] disputed this interpretation and presented additional analyses supporting the robustness of the central conclusions while acknowledging limitations and areas requiring methodological refinement.

This debate is important because it illustrates the central principle of the present Review: mitochondrial variants are not automatically equivalent to conventional irreversible nuclear lineage marks. Heteroplasmy can pre exist, drift, segregate and undergo selection. The usefulness of a mitochondrial variant as a barcode therefore depends not only on detectability but also on its clonal distribution, molecular support and biological context. High confidence lineage reconstruction should ideally exploit multiple concordant mtDNA variants, independent clonal information or analytical procedures that explicitly model heteroplasmic dynamics [16–18].

A useful conceptual boundary nonetheless remains. When an mtDNA variant faithfully identifies descendants without measurably altering their relative success, it functions primarily as a barcode. In that state, the variant can tell us which cells share ancestry, but not why one lineage became larger than another. This distinction is essential whenever lineage identity is correlated with transcriptional or phenotypic state: the mtDNA variant may tag the clone carrying the relevant nuclear, epigenetic or developmental programme without causing that programme itself.

The passenger state: clonal expansion can expose cryptic mtDNA mutations

The strongest recent evidence for a predominantly passenger model comes from large scale human population genetics. Gupta et al. [19] analysed blood whole genome sequencing from approximately 750,000 individuals and showed that heteroplasmic mtDNA single nucleotide variants accumulate sharply with older age, generally occur at low heteroplasmy and exhibit little evidence of positive selection. Their mutational spectrum was more compatible with replication error than with oxidative lesions as a dominant mechanism. Importantly, mitochondrial variant burden associated with germline loci previously connected to clonal haematopoiesis and with somatic mutations in recognised CH driver genes.

These observations support a parsimonious two stage model. A haematopoietic cell first acquires a low abundance mtDNA variant through stochastic mutation. At the population level this variant is effectively cryptic. If that cell subsequently gives rise to an expanding haematopoietic clone, its descendants amplify the mitochondrial sequence signal until the variant becomes detectable in bulk blood. The mitochondrial variant has therefore increased in observed abundance without necessarily having contributed to the selective event responsible for cellular expansion [19].

This behaviour is analogous to genetic hitchhiking. A neutral allele can rise in frequency because it resides in a genetic background being positively selected for another reason. In clonal haematopoiesis, the principal cellular fitness advantage may be conferred by a nuclear driver, whereas one or several mitochondrial variants travel with the expanding lineage. The observation is also consistent with broader studies of somatic mtDNA mosaicism showing frequent stochastic acquisition of mitochondrial variants and replication associated mutational processes in normal human cells, including HSPCs [19, 20].

The passenger model explains why mitochondrial heteroplasmy and clonal haematopoiesis can become increasingly correlated with age without requiring positive selection for each mitochondrial variant. Higher mitochondrial mutation burden can become a sensitive marker of somatic mosaicism because clonally expanding cells reveal otherwise undetectable mitochondrial variants. This interpretation does not make passenger variants biologically uninformative. A neutral passenger can retain considerable value as a record of clone history, expansion or age.

Crucially, the population level findings describe the distribution of effects across very large numbers of mitochondrial variants; they do not establish that every variant is neutral. A predominantly neutral distribution is entirely compatible with a minority of variants undergoing negative selection, altering cellular phenotype or modifying the fitness of specific nuclear clones. The evolutionary task is therefore not to choose universally between “passenger” and “functional”, but to identify the evidence that distinguishes one state from another.

When a passenger becomes biologically consequential

Human mitochondrial disease provides direct evidence that mitochondrial genotype can alter the representation of haematopoietic cells. Walker et al. [10] demonstrated marked depletion of the pathogenic m.3243A > G variant within human T cells, consistent with purifying selection acting against cells carrying high levels of the mutant mitochondrial genome. Franklin et al. [11] subsequently demonstrated that this negative selection becomes progressively stronger during T cell differentiation, linking cellular developmental state to the intensity of mitochondrial selection. Single cell multiomic analysis of individuals with pathogenic mtDNA deletions similarly revealed cell type dependent purifying selection and genotype associated transcriptional responses across immune and haematopoietic compartments [12].

These observations should not be extrapolated uncritically to common low level heteroplasmies. Severe pathogenic mitochondrial variants occupy the deleterious tail of the fitness distribution and may

therefore behave very differently from the majority of age accumulated mtDNA variants. Their importance is conceptual: they prove in humans that mitochondrial genotype can alter cellular representation and that its selective effect can be lineage dependent [10–12].

Population genetic evidence reaches the same principle from another direction. Lake et al. [9] developed a mitochondrial constraint model and showed strong depletion of expected deleterious variation across the human mitochondrial genome, demonstrating that mutational opportunity and observed variation differ in patterns consistent with selection. Thus, mitochondrial variants cannot be treated as a biologically uniform class of neutral markers. A plausible continuum extends from effectively neutral variants through mildly deleterious or context dependent variants to strongly selected pathogenic genotypes.

The critical unresolved question is whether modest mitochondrial differences, well below the severity associated with classical mitochondrial disease, can alter HSPC competition sufficiently to influence the trajectory of an independently initiated haematopoietic clone.

Heteroplasmy and risk of myeloid neoplasia

Hong et al. [21] examined mitochondrial heteroplasmy together with clonal haematopoiesis in a large population cohort and identified several clinically provocative relationships. Heteroplasmy was more common in individuals with clonal haematopoiesis, particularly among those with larger nuclear clone sizes, multiple driver mutations and spliceosome associated mutations. Individuals carrying both clonal haematopoiesis and mitochondrial heteroplasmy had a greater subsequent risk of myeloid neoplasia than individuals with either feature alone, and incorporation of heteroplasmy improved the sensitivity of an existing clonal haematopoiesis risk model. Predicted mitochondrial variant deleteriousness also contained prognostic information.

At least three interpretations remain plausible. Heteroplasmy may simply record an older or more extensively expanded nuclear clone, in which case it functions predominantly as a historical passenger or biomarker. Alternatively, clonal haematopoiesis and mitochondrial heteroplasmy may share upstream determinants such as stem cell turnover, replicative history or altered genomic maintenance. The most provocative interpretation is that particular mitochondrial states influence the subsequent behaviour of nuclear mutant clones.

The available observational evidence cannot distinguish these possibilities definitively. Association with larger clones and multiple nuclear mutations can itself be explained by the passenger model. Risk prediction also does not establish causality: a biomarker can forecast progression without contributing mechanistically to that progression. Nevertheless, the association between predicted mitochondrial variant deleteriousness and clinical risk, together with driver specific patterns, makes complete biological neutrality an insufficient working assumption for every variant [21].

A useful intermediate category is therefore needed. A mitochondrial variant may exert too little effect to initiate detectable clonal expansion by itself yet still alter the survival, self renewal, differentiation or competitive trajectory of a clone driven primarily by a nuclear mutation. We refer to this state as a fitness modifier.

The fitness modifier state

A fitness modifier changes the magnitude or direction of selection acting on an existing cellular clone without necessarily initiating that clone independently. This category is biologically plausible because mitochondrial metabolism and signalling participate directly in HSC fate determination, integrating oxidative phosphorylation, metabolite availability, redox signalling, apoptosis and epigenetic regulation [22, 23]. Nuclear genetic control of mitochondrial copy number and heteroplasmy further demonstrates that mitochondrial state is already embedded within a larger intergenomic regulatory system rather than operating as an isolated cytoplasmic variable [6].

Experimental evidence establishes that mitochondrial activity is not merely a passive correlate of HSC state. Vannini et al. [22] demonstrated that deliberate modulation of mitochondrial activity altered HSC self renewal and differentiation, providing a causal connection between mitochondrial metabolic state and haematopoietic stem cell fate. These observations support the broader concept that mitochondrial state can modify the competitive properties of a haematopoietic cell without itself constituting an initiating somatic driver.

The strongest recent example in clonal haematopoiesis comes from a nuclear CH driver rather than an mtDNA mutation itself. Gozdecka et al. [24] performed a genome wide CRISPR vulnerability screen in *Dnmt3a* R882 mutant HSPCs and identified a substantial enrichment of mitochondrial metabolic dependencies. Mutant cells displayed increased oxidative phosphorylation, and pharmacological inhibition of selected mitochondrial pathways suppressed post transplantation expansion of *Dnmt3a* mutant HSCs more strongly than wild type cells. Metformin reproduced this selective suppression in experimental models, including a primary human *DNMT3A* R882 CH sample. Analysis of 412,234 UK Biobank participants additionally found a markedly lower prevalence of *DNMT3A* R882 clonal haematopoiesis among metformin users after adjustment for relevant confounders.

This study does not establish that mtDNA heteroplasmy causes *DNMT3A* R882 clonal expansion. Its conceptual contribution is subtler: the fitness effect of a nuclear driver can depend on mitochondrial physiology [24]. Nuclear mutations therefore need not possess fixed selection coefficients independent of mitochondrial state. If genetically determined differences in mitochondrial function can modify this dependency, mitochondrial genotype and heteroplasmy become plausible contributors to variation among apparently similar nuclear clones.

The relevant evolutionary question consequently changes. Instead of asking whether an mtDNA variant is independently “oncogenic”, one can ask whether a mitochondrial genotype changes the effective

selection coefficient of the nuclear clone in which it resides. This distinction allows biologically consequential mitochondrial variation to be considered without prematurely labelling it a clonal driver.

Mitonuclear epistasis: two genomes, one fitness phenotype

The mitochondrial genome encodes only a small subset of proteins required for oxidative phosphorylation, whereas the machinery responsible for mtDNA replication and maintenance is nuclear encoded. Large population studies further demonstrate that common and rare nuclear genetic variation measurably influences mtDNA copy number and heteroplasmy in humans [6]. The phenotype commonly described as “mitochondrial” is therefore intrinsically conditioned by coordinated nuclear and mitochondrial biology [6, 25].

This provides a natural basis for mitonuclear epistasis in somatic haematopoiesis. In genetic terms, epistasis occurs when the phenotypic effect of one genetic variant depends on the state of another locus. Applied across genomes, a nuclear CH mutation could create a cellular state in which a mitochondrial variant that was previously nearly neutral becomes advantageous or deleterious. Conversely, a pre existing mitochondrial genotype could alter the cellular background into which a nuclear driver subsequently arises [25].

Three biologically distinct forms of interaction can be envisaged. A nuclear driver may create a mitochondrial dependency, as demonstrated experimentally for *DNMT3A* R882 [24]. A mitochondrial genotype may alter the selective value of a nuclear mutation by changing respiratory reserve, metabolite availability, redox state or apoptotic threshold. Finally, selection may act primarily on the combined mitonuclear genotype, enriching or depleting particular genomic combinations rather than either variant considered independently [23, 25].

These models remain hypotheses for mtDNA heteroplasmy in CH and should be treated as such. Their value is that they generate experimentally distinguishable predictions. If mitochondrial background is irrelevant, nuclear driver fitness should be similar across otherwise matched mitochondrial states. If epistasis is present, the effect of one genome should differ according to the genotype of the other. The evolutionary unit would then be the cell carrying a particular combination of nuclear and mitochondrial genomes, not either genome in isolation.

Conceptually, clonal fitness can therefore be represented as $\text{clonal fitness} = f(\text{nuclear genotype, mitochondrial genotype, heteroplasmy, lineage, cellular environment, age})$. The expression is not intended as a complete quantitative model. It emphasises that assigning a single intrinsic fitness value to a nuclear mutation may obscure biologically relevant genomic context. Longitudinal CH studies already demonstrate gene and allele specific differences in clone growth, but considerable heterogeneity remains among apparently similar clones [3, 4]. Nuclear co mutations, germline background, inflammatory state and timing explain part of that variation. Mitochondrial genotype is a plausible additional dimension requiring direct experimental testing.

Barcode, passenger, fitness modifier or driver: a proposed classification

The available evidence supports a four state framework for mitochondrial heteroplasmy in haematopoiesis, with a mitonuclear interaction state superimposed across these categories. The defining biological roles, expected observations, evidentiary requirements and principal interpretative hazards of these states are summarised in Table 1.

Table 1

Proposed evolutionary states of mitochondrial heteroplasmy in haematopoiesis, with defining biological roles, expected observations, evidentiary requirements and principal interpretative risks

State	Biological role	Expected observation	Evidence required	Principal interpretative risk
Barcode	Records cellular ancestry	Variant tracks a lineage or clone	Concordant clonal markers, adequate molecular support and negligible detectable fitness effect	Attributing the phenotype of the tagged clone to the barcode
Passenger	Hitchhikes with an expanding cellular clone	Bulk heteroplasmy increases with clone size	An independent driver explains cellular expansion, with little evidence for positive selection on mtDNA	Mistaking correlation with clonal expansion for mtDNA causality
Fitness modifier	Alters the selection coefficient of an existing clone	Clonal behaviour differs according to mitochondrial state	Isogenic or longitudinal evidence of genotype dependent competitive effects	Mistaking modification of clonal fitness for autonomous clone initiation
Driver	Directly confers a cellular competitive advantage	mtDNA genotype precedes and sustains clonal expansion	Recurrent selection, temporal precedence and experimental sufficiency	Misclassifying a biomarker or hitchhiking variant as a driver
Mitonuclear epistatic state	Nuclear and mitochondrial genotypes jointly determine cellular fitness	Effect of one genome depends on the state of the other	Factorial genotype experiments together with longitudinal human validation	Analysing two interacting genomes independently

Legend. The proposed framework distinguishes mitochondrial DNA variants according to the evolutionary role they may assume within a haematopoietic clone. A barcode records cellular ancestry without materially altering fitness, whereas a passenger increases in observed abundance because the cellular clone carrying it expands for an independent reason. A fitness modifier changes the competitive behaviour of an existing clone without necessarily initiating clonal expansion. Driver status is reserved for mitochondrial alterations demonstrated to confer a direct cellular selective advantage. A mitonuclear epistatic state denotes a context in which the biological or fitness effect of either genome depends on the state of the other. These categories are not necessarily immutable properties of individual variants and may vary according to nuclear genotype, heteroplasmy level, lineage, developmental state and cellular context.

Abbreviation: mtDNA, mitochondrial DNA.

A barcode is an mtDNA variant that conveys information about cellular ancestry while exerting negligible influence on the clonal behaviour being reconstructed. Confidence in this interpretation increases when multiple concordant variants, independent clonal markers or robust molecular support reproduce the

inferred architecture. The ongoing methodological debate around mitochondrial single cell phylogenies demonstrates why barcode status must be empirically supported rather than assumed [13, 16–18].

A passenger is a variant whose measured abundance rises because the cellular clone carrying it expands for another reason. The population pattern reported by Gupta et al. [19] strongly supports this explanation for many age associated blood mtDNA variants. Passenger status is particularly plausible when low level variants accumulate without evidence of positive mitochondrial selection and accompany known nuclear CH drivers.

A fitness modifier changes the magnitude or direction of clonal selection but does not independently explain clone initiation. Evidence would require demonstration that cellular competitive behaviour differs according to mitochondrial genotype or heteroplasmy after controlling for nuclear genotype. The genotype specific mitochondrial metabolic dependence of *DNMT3A* R882 CH establishes the plausibility of this class, although direct demonstration that naturally occurring mtDNA heteroplasmy provides such modification remains an important experimental gap [24].

A driver should represent the most stringent category. An mtDNA alteration would need to confer sufficient cellular advantage to initiate or maintain clonal expansion independently of known nuclear drivers. High heteroplasmy, statistical association, predicted deleteriousness or enrichment in individuals with CH is not sufficient evidence. Convincing driver designation would require recurrent enrichment beyond mutational expectation, temporal precedence, cellular rather than solely intracellular selection, and experimental demonstration that the mitochondrial genotype itself increases competitive fitness.

A fifth state, mitonuclear epistasis, need not be mutually exclusive with the four categories. A mitochondrial variant that behaves as a passenger in one nuclear background could become a fitness modifier in another. The classification is therefore context dependent rather than permanently attached to a sequence variant [24, 25].

Selection occurs at more than one biological level

A major source of confusion is that mitochondrial evolution operates simultaneously at multiple biological levels. Within a cell, mutant and wild type mitochondrial genomes can change in relative abundance through replication, degradation, segregation and stochastic drift. Between cells, mitochondrial genotype can affect survival, proliferation or differentiation. Among HSC clones, nuclear and mitochondrial states may jointly influence competitive success. During differentiation, lineage specific metabolic demands can impose additional selection [5, 8, 10–12].

A rise in population level heteroplasmy therefore does not identify the level at which selection occurred. Consider an HSC carrying a mitochondrial variant at low heteroplasmy that subsequently acquires, or already contains, a positively selected nuclear CH driver. Years later the clone occupies a large fraction of blood cells and the mitochondrial variant becomes readily detectable in bulk sequencing. This

observation is compatible with several distinct histories: the mitochondrial fraction may have remained approximately stable while the cellular clone expanded; intracellular heteroplasmy may have drifted upwards during clonal expansion; high heteroplasmy subclones may have gained or lost additional cellular fitness; or different blood lineages may have selected different heteroplasmy states.

Bulk blood sequencing cannot distinguish these histories reliably. The phrase “selection of an mtDNA mutation” should therefore be used only after the level of selection has been identified. A mitochondrial genome can increase within cells while the cells themselves are being negatively selected, and a mitochondrial sequence signal can increase in bulk blood even when its intracellular fraction is unchanged, simply because the cellular lineage carrying it has expanded.

Lineage is part of the genotype–fitness relationship

Haematopoiesis provides an unusually informative system for studying context dependent mitochondrial selection because differentiated blood lineages have distinct metabolic programmes. Evidence from mitochondrial disease demonstrates strong negative selection against pathogenic mtDNA during T cell differentiation, whereas other haematopoietic compartments can tolerate the same genotype differently [10–12].

Clone fitness therefore cannot always be inferred from whole blood abundance. A mutation bearing HSC can persist within the stem cell compartment while its high heteroplasmy progeny are strongly depleted from mature T cell populations. Conversely, a mitochondrial state that is largely tolerated in a quiescent HSC may become disadvantageous during rapid proliferative differentiation. This issue is especially relevant to clonal haematopoiesis because nuclear CH drivers themselves can exhibit distinct lineage distributions and biases [26].

A mitochondrial genotype that impairs lymphoid differentiation while sparing myeloid production could consequently appear to favour a myeloid biased nuclear clone even without directly increasing HSC self renewal. Apparent clonal selection may therefore partly reflect differential lineage output, not only differential stem cell abundance. Future studies should simultaneously determine nuclear genotype, mitochondrial genotype and heteroplasmy across purified HSCs, intermediate progenitors and mature blood lineages. The principal studies underpinning the barcode, passenger and fitness modifier framework, together with their major methodological and biological limitations, are summarised in Table 2.

Table 2

Key studies informing the barcode, passenger and fitness modifier framework, with experimental systems, principal observations, evolutionary interpretation and major limitations

Study	Experimental or clinical system	Principal observation	Evolutionary interpretation	Important limitation
Ludwig et al. [13]	Human single cells	Somatic mtDNA variants can reconstruct cellular relationships together with cellular state information	Supports the use of mtDNA as an endogenous lineage barcode	Functional neutrality and clonal informativeness vary among variants
Lareau et al. [14]	Single cell mtDNA and chromatin profiling	High throughput mitochondrial genotyping resolves clonal relationships alongside epigenomic state	Extends mtDNA barcode utility	Association between a barcode and cellular state does not establish causality
Walker et al. [10]	Human mitochondrial disease	Pathogenic heteroplasmy is depleted in T cells	Direct evidence of lineage specific purifying selection	Severe pathogenic variants may not model common low level heteroplasmy
Franklin et al. [11]	Human T cell differentiation	Negative selection against pathogenic mitochondrial variants strengthens during differentiation	Demonstrates developmental state dependence of mitochondrial selection	Disease associated variants occupy the deleterious end of the heteroplasmy spectrum
Lareau et al. [12]	Human mtDNA deletion disorders	Cell type dependent purifying selection is accompanied by transcriptional adaptation	Demonstrates that mitochondrial genotype can alter cellular representation	Evidence derives from rare mitochondrial disease

Legend. Selected studies illustrate the progression from mitochondrial DNA as an endogenous lineage marker to investigation of its potential participation in clonal biology. Current evidence strongly supports the utility of high confidence mtDNA variants for lineage tracing and favours a predominantly passenger interpretation for many age associated blood heteroplasmies. Experimental and human data nevertheless establish biological plausibility for context dependent mitochondrial modification of clonal fitness. Direct evidence that naturally occurring mtDNA heteroplasmy independently initiates or drives clonal haematopoiesis remains limited. Methodological controversies concerning low support mitochondrial variants and phylogenetic reconstruction are included because they directly influence the evidentiary threshold required for assigning barcode status.

Study	Experimental or clinical system	Principal observation	Evolutionary interpretation	Important limitation
Weng et al. [15]	Human haematopoiesis	Deep mtDNA profiling suggests clonally resolved HSC output and age related oligoclonality	Demonstrates the potential for high resolution endogenous lineage barcoding	Aspects of variant calling and phylogenetic inference remain contested
Wang et al. [16]	Computational modelling and single cell genomics	Lineage tracing performance improves with strong clonal expansion and can be confounded by pre existing heteroplasmy	Defines biological and analytical conditions required for informative mtDNA barcoding	Generalisability depends on biological context and assay performance
Hong et al. [21]	Population cohort	Heteroplasmy associates with high risk CH features and improves prediction of myeloid neoplasm risk	Compatible with passenger, biomarker or fitness modifier states	Observational association cannot establish mitochondrial causality
Gozdecka et al. [24]	Mouse and human HSPCs with UK Biobank validation	Fitness of <i>DNMT3A</i> R882 mutant clones depends on mitochondrial metabolism	Provides mechanistic evidence for nuclear genotype specific mitochondrial dependency	Metabolic dependency does not establish causality for mtDNA heteroplasmy itself
Gupta et al. [19]	Approximately 750,000 human blood genomes	Many age accumulated mtDNA SNVs show little evidence of positive selection and become detectable with clonal expansion	Strongly supports a predominant passenger model	Population level effects may obscure rare functional variants
Lareau et al. [17];	Methodological reanalysis and author response	Debate centres on low support mtDNA variants	Defines an important methodological	Does not invalidate the broader utility of high confidence

Legend. Selected studies illustrate the progression from mitochondrial DNA as an endogenous lineage marker to investigation of its potential participation in clonal biology. Current evidence strongly supports the utility of high confidence mtDNA variants for lineage tracing and favours a predominantly passenger interpretation for many age associated blood heteroplasmies. Experimental and human data nevertheless establish biological plausibility for context dependent mitochondrial modification of clonal fitness. Direct evidence that naturally occurring mtDNA heteroplasmy independently initiates or drives clonal haematopoiesis remains limited. Methodological controversies concerning low support mitochondrial variants and phylogenetic reconstruction are included because they directly influence the evidentiary threshold required for assigning barcode status.

Study	Experimental or clinical system	Principal observation	Evolutionary interpretation	Important limitation
Weng et al. [18]		and robustness of inferred phylogenies	boundary for barcode interpretation	mtDNA lineage markers
Legend. Selected studies illustrate the progression from mitochondrial DNA as an endogenous lineage marker to investigation of its potential participation in clonal biology. Current evidence strongly supports the utility of high confidence mtDNA variants for lineage tracing and favours a predominantly passenger interpretation for many age associated blood heteroplasmies. Experimental and human data nevertheless establish biological plausibility for context dependent mitochondrial modification of clonal fitness. Direct evidence that naturally occurring mtDNA heteroplasmy independently initiates or drives clonal haematopoiesis remains limited. Methodological controversies concerning low support mitochondrial variants and phylogenetic reconstruction are included because they directly influence the evidentiary threshold required for assigning barcode status.				

Abbreviations: CH, clonal haematopoiesis; HSC, haematopoietic stem cell; HSPC, haematopoietic stem and progenitor cell; mtDNA, mitochondrial DNA; SNV, single nucleotide variant.

Why clone size and heteroplasmy are easy to confuse

Variant allele fraction is already an imperfect surrogate for cellular clone size in nuclear clonal haematopoiesis. Mitochondrial heteroplasmy introduces another layer because bulk mitochondrial allele fraction reflects at least four quantities: the fraction of blood cells belonging to a clone, the intracellular fraction of mitochondrial genomes carrying the variant, mitochondrial copy number and the lineage composition of the sampled blood.

Two individuals with identical measured bulk heteroplasmy can therefore have very different biological architectures. One may have a small cellular clone in which each cell contains a high mutant mitochondrial fraction; another may have a very large clone in which each cell carries only low level heteroplasmy. Those states should not be assumed to have equivalent evolutionary or physiological consequences.

Mitochondrial copy number further varies among cell types, and blood composition itself can confound bulk mitochondrial measurements. Gupta et al. [6] demonstrated that many previously reported associations between blood mtDNA copy number and disease changed substantially after correction for cellular composition, highlighting how apparent mitochondrial phenotypes can emerge from shifts in the proportions of blood cell populations rather than mitochondrial biology alone.

Evolutionary studies should therefore measure, wherever feasible, cellular clone fraction, single cell heteroplasmy distribution, mtDNA copy number, nuclear driver genotype and lineage identity. A single bulk heteroplasmy value collapses these biologically distinct dimensions and should not be interpreted as a direct measure of mitochondrial or cellular fitness.

Distinguishing causation from clonal hitchhiking

The central methodological challenge is to separate mitochondrial causality from clonal hitchhiking. Cross sectional association is insufficient, and even longitudinal increases in heteroplasmy can mislead if the cellular clone carrying the variant expands because of an independent nuclear driver [19, 21]. Strong inference will require experimental designs in which the two genomes can be interrogated separately.

The most informative approach would be factorial perturbation. Otherwise matched HSPCs could be constructed with wild type or mutant nuclear states crossed against defined mitochondrial states. Competitive culture or transplantation could then estimate the effect of the nuclear driver, the mitochondrial genotype and, critically, the interaction between them. A significant interaction would constitute direct evidence of mitonuclear epistasis rather than simple additive mitochondrial dysfunction.

Heteroplasmy should also be treated quantitatively rather than as presence or absence. Experimentally generating a range of heteroplasmy levels would reveal whether a mitochondrial variant has a linear effect, a threshold, a non monotonic optimum or no measurable fitness consequence. This is particularly important because established mitochondrial diseases frequently display threshold dependent phenotypes [8].

Longitudinal single cell phylogenies offer a complementary human approach. Repeated samples integrating nuclear driver genotype, high confidence mtDNA heteroplasmy and cellular state could determine whether mitochondrial changes precede, accompany or merely follow alterations in nuclear clone growth. Recent debates around mtDNA lineage inference emphasise that such analyses should incorporate stringent variant support, multiple markers and, where possible, orthogonal lineage information [16–18].

Inherited mitochondrial variation provides another potentially informative natural experiment because mitochondrial genotype can precede acquisition of somatic nuclear drivers by decades. Large biobanks integrating inherited mitochondrial haplotypes or heteroplasms with longitudinal CH could ask whether pre existing mitochondrial backgrounds alter the probability that particular nuclear drivers subsequently expand. The observation that nuclear loci control mtDNA abundance and heteroplasmy further suggests that the reciprocal direction of interaction should also be incorporated [6].

Finally, mitochondrial genome editing increasingly makes direct perturbation technically conceivable. DddA derived mitochondrial base editors enabled targeted C to T changes without CRISPR guide RNA delivery, and TALE linked deaminases subsequently extended programmable mitochondrial editing to A to G substitutions [27, 28]. These tools are not yet a routine platform for competitive human HSC evolution experiments, and off target activity, editing scope and model specific constraints remain important. Nevertheless, they shift a previously observational field towards the possibility of directly introducing or correcting selected mitochondrial genotypes while holding the nuclear background constant. The experimental strategies capable of separating lineage marking, clonal hitchhiking, fitness modification and genuine mitochondrial causality are outlined in Table 3.

Table 3

Experimental strategies for distinguishing lineage marking, clonal hitchhiking, fitness modification and autonomous driver activity of mitochondrial heteroplasmy

Biological question	Preferred experimental design	Result supporting causal interpretation
Does the variant function only as a lineage marker?	mtDNA lineage tracing with orthogonal nuclear or independent clonal markers	Concordant lineage structure without genotype dependent differences in cellular growth or competitive fitness
Is the variant hitchhiking with a nuclear driver?	Longitudinal paired nuclear and mitochondrial genotyping	Mitochondrial signal follows expansion of the cellular clone without evidence of within clone selection for the mtDNA variant
Does heteroplasmy alter cellular fitness?	Isogenic heteroplasmy titration	Reproducible heteroplasmy dependent alteration in competitive cellular fitness
Does the effect depend on CH genotype?	Factorial nuclear driver × mitochondrial genotype experiment	Statistically significant interaction between nuclear and mitochondrial states
Does lineage alter mitochondrial selection?	Single cell analysis across HSC, progenitor and mature cell compartments	Direction or magnitude of selection on the mitochondrial variant differs among haematopoietic lineages or developmental compartments
Can mtDNA independently initiate clonal expansion?	Longitudinal human cohorts combined with experimentally engineered competition models	mtDNA alteration precedes clonal expansion and independently increases competitive cellular fitness
Legend. The central experimental challenge is to distinguish mitochondrial causality from clonal hitchhiking. Orthogonal lineage validation is required to establish barcode function, whereas paired longitudinal nuclear and mitochondrial genotyping can determine whether an mtDNA variant merely accompanies expansion driven by an independent nuclear lesion. Isogenic heteroplasmy titration and factorial mitonuclear experiments are required to establish direct or interaction dependent effects on cellular fitness. Lineage resolved analysis is necessary because mitochondrial selection may differ among haematopoietic compartments. Assignment of autonomous driver status should require both temporal precedence and experimental demonstration that the mtDNA alteration itself increases competitive cellular fitness.		

Abbreviations: CH, clonal haematopoiesis; HSC, haematopoietic stem cell; mtDNA, mitochondrial DNA.

A driver should require a high evidentiary bar

The term “driver” is appropriately powerful in cancer genetics and should be used even more cautiously for mitochondrial heteroplasmy. The multicopy nature of mtDNA, high mutation rate, intracellular drift, lineage dependent selection and clonal hitchhiking can generate patterns that superficially resemble positive cellular selection even when the mitochondrial variant is not responsible for expansion.

An mtDNA variant should therefore not be considered a haematopoietic clonal driver simply because its heteroplasmy increases with age, because it is enriched in individuals with clonal haematopoiesis, because it predicts myeloid neoplasia, because it reaches high intracellular abundance or because

computational models predict functional deleteriousness [19, 21]. Each observation may strengthen biological interest, but none alone establishes positive selection of mutation bearing cells.

A persuasive driver claim should ideally converge across three levels of evidence. Population evidence should show enrichment beyond what is expected from mutational opportunity and hitchhiking. Clonal evidence should demonstrate that cells carrying the variant preferentially expand, rather than merely that mutant mtDNA molecules increase within existing cells. Functional evidence should show that manipulating the mitochondrial genotype changes competitive cellular fitness while relevant nuclear variables are controlled. Until these conditions are met, “fitness modifier” is often the more scientifically defensible term.

Reconciling seemingly conflicting evidence

The passenger and functional models are not mutually exclusive. Gupta et al. [19] provide compelling evidence that the majority of age accumulated mtDNA SNVs detectable in human blood are likely neutral or nearly neutral variants exposed by age related clonal expansion. This is a statement about the overall distribution of mitochondrial fitness effects. It does not preclude a smaller subset from being selected.

Human mitochondrial disease establishes that some mitochondrial genotypes experience powerful negative cellular selection, particularly in defined immune lineages [10–12]. Constraint analysis independently demonstrates that deleterious mitochondrial variation is depleted from human populations [9]. Hong et al. [21] show that heteroplasmy adds prognostic information to nuclear CH variables even though the mechanism of that association remains unresolved. Gozdecka et al. [24] then demonstrate that mitochondrial physiology can be a genotype specific determinant of nuclear CH clone fitness.

These findings are readily reconciled by a distribution of mitochondrial fitness effects dominated by near neutral variation but containing a smaller tail of context dependent functional variants. The appropriate scientific objective is therefore not to determine whether “mtDNA heteroplasmy” as a class is neutral or causal. The objective is to identify the rules by which a particular mitochondrial state occupies one evolutionary category in one cellular context and another category elsewhere.

From a fixed fitness landscape to a conditional fitness landscape

The fitness landscape concept has been highly productive in clonal haematopoiesis. Population modelling demonstrates that positive selection, rather than drift alone, is a dominant force shaping detectable CH, while longitudinal studies show that different nuclear drivers produce markedly different rates of expansion [3, 4]. These observations are often interpreted as gene or mutation specific fitness effects.

A mitonuclear framework suggests that these fitness effects may be conditional rather than fixed. A nuclear driver may possess one effective selection coefficient in an HSPC with robust mitochondrial

respiratory capacity and another in a cell with altered mitochondrial genotype or constrained metabolic reserve. Likewise, the same mitochondrial variant may be effectively neutral in one nuclear background and deleterious or advantageous in another [24, 25].

The conceptual fitness relationship can therefore be represented as $s = f(N, M, H, L, E, t)$, where N denotes nuclear genotype, M mitochondrial genotype, H heteroplasmy, L lineage or cellular state, E cellular environment and t age or time. This formulation is not proposed as a fitted quantitative model; it is a reminder that the observed growth of a clone emerges from multiple interacting variables.

This view also shifts the interpretation of heterogeneity among clones carrying the same nuclear driver. Differences in nuclear allele, co mutations, germline background, inflammatory exposure, acquisition time and stochastic history remain important [3, 4]. Mitochondrial genotype and heteroplasmy should be added as testable, rather than assumed, contributors to that unexplained variation.

Ageing as the common axis

Ageing connects nearly every component of this framework. Somatic nuclear mutations accumulate, clonal diversity changes, mtDNA variation accumulates, mitochondrial function shifts, cellular composition changes and the bone marrow environment itself evolves [29, 30]. Selection consequently acts upon increasing genetic variation within a changing biological landscape.

Mitochondrial lineage studies suggest substantial age associated remodelling of haematopoietic clonal structure, although the exact resolution and reliability of specific phylogenetic approaches remain under active methodological discussion [15, 17, 18]. Independently, population sequencing demonstrates a striking increase in detectable heteroplasmic mtDNA SNVs at older ages and strongly links this phenomenon to haematopoietic clonal expansion [19]. Normal somatic cell sequencing likewise demonstrates stochastic accumulation of mitochondrial mosaicism with age and strong signatures of replication associated mutagenesis [20].

Mitochondrial state itself may contribute to age associated heterogeneity of the HSC compartment. Mansell et al. [29] demonstrated that mitochondrial membrane potential identifies functionally distinct aged HSC populations and that experimental enhancement of mitochondrial function can alter lineage output and improve aspects of aged HSC performance. Such findings reinforce the possibility that ageing modifies not only the abundance of mitochondrial variants but also the cellular context in which their phenotypic consequences are expressed.

Together, these findings suggest that ageing does more than generate additional mitochondrial variants. It provides the cellular clonal expansions required to reveal many of them. A deeper possibility is that ageing may also alter the fitness consequences of selected heteroplasmies through changes in respiratory reserve, mitophagy, stem cell cycling, lineage output, inflammation and remodelling of the bone marrow niche [29, 30]. Longitudinal studies beginning before overt clonal haematopoiesis will

therefore be more informative than late life cross sectional comparisons for distinguishing cause from consequence.

Implications for risk prediction

Mitochondrial heteroplasmy may prove clinically useful even if many variants are passengers. A predictive biomarker does not need to be mechanistically causal. If heteroplasmy records clone age, expansion history, cellular turnover or another dimension not captured completely by nuclear variant allele fraction, it can improve risk stratification while remaining evolutionarily neutral.

Hong et al. [21] provide proof of principle by showing that mitochondrial heteroplasmy improves identification of individuals with clonal haematopoiesis at higher risk of myeloid neoplasia. The interpretation should nevertheless separate prediction from intervention. A passenger can be highly predictive yet represent a poor therapeutic target. A fitness modifier can be predictive and mechanistically actionable. A true driver would offer the strongest direct rationale for genotype specific intervention.

The experimental work on *DNMT3A* R882 further suggests that mitochondrial pathways could be therapeutically relevant even when the causal lesion is nuclear. Selectively attenuating a mitochondrial dependency created by a high risk nuclear clone could represent a form of evolutionary interception, reducing the competitive advantage of the premalignant clone before additional transforming events accumulate [24]. Whether such an approach is clinically feasible or safe remains unresolved, but the conceptual shift is important: mitochondria need not contain the initiating mutation to become part of an evolutionarily targeted intervention.

The next experimental agenda

The most important unresolved question is which naturally occurring mtDNA variants are genuinely neutral within human HSPCs. Large population studies can estimate mutational spectra and infer selection across hundreds of thousands of individuals, but population averages may conceal rare variants with substantial cellular effects [19]. High confidence single cell and clonal assays are required to determine whether variants classified as broadly neutral remain neutral within defined stem cell and lineage contexts.

A second priority is to determine whether mitochondrial selection is nuclear driver specific. The relationships between heteroplasmy and spliceosome mutant CH reported by Hong et al. [21] are particularly intriguing, but observational enrichment should not be treated as evidence of epistasis. Equivalent experiments should test *DNMT3A*, *TET2*, *ASXL1*, *JAK2*, *TP53* and spliceosome driver backgrounds directly.

Third, heteroplasmy should be studied as a continuous variable. A mitochondrial variant may be neutral below a threshold, deleterious above it, or display different effects across lineages [8]. Experiments

based only on presence versus absence are poorly suited to this biology.

Fourth, inherited mitochondrial variation provides a largely untapped opportunity to test temporal precedence. Because mitochondrial genotype can be present decades before acquisition of a somatic nuclear CH driver, longitudinal biobank studies can investigate whether pre existing mitochondrial backgrounds alter the probability that specific nuclear clones subsequently expand. Nuclear genetic control of mtDNA heteroplasmy also means that reciprocal interaction should be assessed rather than presuming one directional causality [6].

Fifth, future lineage studies need to separate stem cell abundance from differentiated output. An mtDNA genotype that alters T cell production but leaves HSC self renewal unchanged should not be described as an HSC clonal driver. Simultaneous sampling of HSCs, progenitors and mature lineages is essential [10–12, 26].

Finally, mitochondrial genome engineering creates the possibility of testing causality directly. Current editing technologies do not yet permit arbitrary manipulation of every mitochondrial sequence in primary human HSCs, but DdCBE and TALED platforms demonstrate that the previously formidable barrier to programmable mtDNA editing is no longer absolute [27, 28]. Combining such technologies with competitive haematopoietic models may eventually provide the decisive experiments needed to move mitochondrial heteroplasmy from association to evolutionary mechanism.

Conclusions

Mitochondrial heteroplasmy occupies an unusual position in human somatic evolution. It can provide a record of cellular ancestry, hitchhike with a nuclear driven clone, capture information about advanced clonal architecture, undergo lineage specific purifying selection and, potentially, modify the competitive behaviour of cells carrying particular nuclear mutations. These roles are biologically distinct and should not be collapsed into a single interpretation.

The evidence is currently asymmetric. Naturally occurring mtDNA variants are established tools for tracing human cellular ancestry, although the reliability of specific lineage reconstruction strategies is now being actively refined. Large scale population genetics strongly supports a predominantly passenger model for many age accumulated blood mtDNA variants. Human mitochondrial disease proves that mitochondrial genotype can alter cellular representation through lineage dependent selection. Population studies show that heteroplasmy adds information to myeloid neoplasm risk, while functional experiments demonstrate that mitochondrial activity can influence HSC fate and that at least one important CH genotype, *DNMT3A* R882, possesses a specific dependence on mitochondrial metabolism.

The next advance will therefore not come from asking whether mitochondrial heteroplasmy is “important” in clonal haematopoiesis. The useful questions are more precise: important in which clone, at what heteroplasmy, in which lineage, with which nuclear genotype, at what age and at which level of

selection? Answering them will require separation of mitochondrial genome dynamics from cellular clone dynamics and direct testing of interactions between nuclear and mitochondrial states.

Human haematopoiesis contains two genetically distinct but biologically interdependent genomes. Treating one solely as evolutionary mechanism and the other solely as evolutionary information may prove an artificial distinction. A more realistic model is one of conditional somatic fitness in which nuclear mutation, mitochondrial variation, heteroplasmy, lineage and cellular state jointly determine which clones persist, expand or disappear. In most instances the mitochondrial variant may prove to be only the barcode or the passenger. The central challenge is to identify the rarer circumstances in which it changes the journey.

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The author conceived the Review, developed the conceptual framework, critically evaluated and interpreted the literature, prepared and revised the manuscript, approved the final version, and accepts full responsibility for the integrity and content of the work.

References

1. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF et al (2014) Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 371:2477–2487. <https://doi.org/10.1056/NEJMoa1409405>
2. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG et al (2014) Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 371:2488–2498. <https://doi.org/10.1056/NEJMoa1408617>
3. Watson CJ, Papula AL, Poon GYP, Wong WH, Young AL, Druley TE et al (2020) The evolutionary dynamics and fitness landscape of clonal hematopoiesis. *Science* 367:1449–1454. <https://doi.org/10.1126/science.aay9333>
4. Robertson NA, Latorre-Crespo E, Terradas-Terradas M, Lemos-Portela J, Purcell AC, Livesey BJ et al (2022) Longitudinal dynamics of clonal hematopoiesis identifies gene-specific fitness effects. *Nat Med* 28:1439–1446. <https://doi.org/10.1038/s41591-022-01883-3>
5. Stewart JB, Chinnery PF (2021) Extreme heterogeneity of human mitochondrial DNA from organelles to populations. *Nat Rev Genet* 22:106–118. <https://doi.org/10.1038/s41576-020-00284-x>
6. Gupta R, Kanai M, Durham TJ, Tsuo K, McCoy JG, Kotrys AV et al (2023) Nuclear genetic control of mtDNA copy number and heteroplasmy in humans. *Nature* 620:839–848. <https://doi.org/10.1038/s41586-023-06426-5>
7. Ryall C, Chinnery PF, van den Amele J (2026) Common principles underlie mitochondrial DNA heteroplasmy dynamics in the germline and soma. *Annu Rev Genom Hum Genet* 27:247–275. <https://doi.org/10.1146/annurev-genom-120324-032239>
8. Nissanka N, Moraes CT (2020) Mitochondrial DNA heteroplasmy in disease and targeted nuclease-based therapeutic approaches. *EMBO Rep* 21:e49612. <https://doi.org/10.15252/embr.201949612>
9. Lake NJ, Ma K, Liu W, Battle SL, Laricchia KM, Tiao G et al (2024) Quantifying constraint in the human mitochondrial genome. *Nature* 635:390–397. <https://doi.org/10.1038/s41586-024-08048-x>

10. Walker MA, Lareau CA, Ludwig LS, Karaa A, Sankaran VG, Regev A et al (2020) Purifying selection against pathogenic mitochondrial DNA in human T cells. *N Engl J Med* 383:1556–1563. <https://doi.org/10.1056/NEJMoa2001265>
11. Franklin IG, Milne P, Childs J, Boggan RM, Barrow I, Lawless C et al (2023) T cell differentiation drives the negative selection of pathogenic mitochondrial DNA variants. *Life Sci Alliance* 6:e202302271. <https://doi.org/10.26508/lsa.202302271>
12. Lareau CA, Dubois SM, Buquicchio FA, Hsieh YH, Garg K, Kautz P et al (2023) Single-cell multi-omics of mitochondrial DNA disorders reveals dynamics of purifying selection across human immune cells. *Nat Genet* 55:1198–1209. <https://doi.org/10.1038/s41588-023-01433-8>
13. Ludwig LS, Lareau CA, Ulirsch JC, Christian E, Muus C, Li LH et al (2019) Lineage tracing in humans enabled by mitochondrial mutations and single-cell genomics. *Cell* 176:1325–1339e22. <https://doi.org/10.1016/j.cell.2019.01.022>
14. Lareau CA, Ludwig LS, Muus C, Gohil SH, Zhao T, Chiang Z et al (2021) Massively parallel single-cell mitochondrial DNA genotyping and chromatin profiling. *Nat Biotechnol* 39:451–461. <https://doi.org/10.1038/s41587-020-0645-6>
15. Weng C, Yu F, Yang D, Poeschla M, Liggett LA, Jones MG et al (2024) Deciphering cell states and genealogies of human haematopoiesis. *Nature* 627:389–398. <https://doi.org/10.1038/s41586-024-07066-z>
16. Wang X, Wang K, Zhang W, Tang Z, Zhang H, Cheng Y et al (2025) Clonal expansion dictates the efficacy of mitochondrial lineage tracing in single cells. *Genome Biol* 26:70. <https://doi.org/10.1186/s13059-025-03540-7>
17. Lareau CA, Chapman MS, Penter L, Nawy T, Pe'er D, Ludwig LS (2026) Artefacts in single-cell mtDNA analyses misinform phylogenies. *Nature* 656:E25–E31. <https://doi.org/10.1038/s41586-026-10777-0>
18. Weng C, Weissman JS, Sankaran VG (2026) Reply to: Artefacts in single-cell mtDNA analyses misinform phylogenies. *Nature* 656:E32–E38. <https://doi.org/10.1038/s41586-026-10776-1>
19. Gupta R, Durham TJ, Chau G, Kanai M, Uddin MM, Lu W et al (2026) Mechanism of age-related accumulation of mtDNA mutations in human blood. *Nature* 656:140–148. <https://doi.org/10.1038/s41586-026-10569-6>
20. An J, Nam CH, Kim R, Lee Y, Won H, Park S et al (2024) Mitochondrial DNA mosaicism in normal human somatic cells. *Nat Genet* 56:1665–1677. <https://doi.org/10.1038/s41588-024-01838-z>
21. Hong YS, Pasca S, Shi W, Puiu D, Lake NJ, Lek M et al (2024) Mitochondrial heteroplasmy improves risk prediction for myeloid neoplasms. *Nat Commun* 15:10133. <https://doi.org/10.1038/s41467-024-54443-3>
22. Vannini N, Girotra M, Naveiras O, Nikitin G, Campos V, Giger S et al (2016) Specification of haematopoietic stem cell fate via modulation of mitochondrial activity. *Nat Commun* 7:13125. <https://doi.org/10.1038/ncomms13125>

23. Chakrabarty RP, Chandel NS (2021) Mitochondria as signaling organelles control mammalian stem cell fate. *Cell Stem Cell* 28:394–408. <https://doi.org/10.1016/j.stem.2021.02.011>
24. Gozdecka M, Dudek M, Wen S, Gu M, Stopforth RJ, Rak J et al (2025) Mitochondrial metabolism sustains DNMT3A-R882-mutant clonal haematopoiesis. *Nature* 642:431–441. <https://doi.org/10.1038/s41586-025-08980-6>
25. Peng B, Wang Y, Zhang H (2025) Mitonuclear communication in stem cell function. *Cell Prolif* 58:e13796. <https://doi.org/10.1111/cpr.13796>
26. Buscarlet M, Provost S, Zada YF, Bourgoin V, Mollica L, Dubé MP, Busque L (2018) Lineage restriction analyses in CHIP indicate myeloid bias for TET2 and multipotent stem cell origin for DNMT3A. *Blood* 132:277–280. <https://doi.org/10.1182/blood-2018-01-829937>
27. Mok BY, de Moraes MH, Zeng J, Bosch DE, Kotrys AV, Raguram A et al (2020) A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. *Nature* 583:631–637. <https://doi.org/10.1038/s41586-020-2477-4>
28. Cho SI, Lee S, Mok YG, Lim K, Lee J, Lee JM et al (2022) Targeted A-to-G base editing in human mitochondrial DNA with programmable deaminases. *Cell* 185:1764–1776e12. <https://doi.org/10.1016/j.cell.2022.03.039>
29. Mansell E, Sigurdsson V, Deltcheva E, Brown J, James C, Miharada K et al (2021) Mitochondrial potentiation ameliorates age-related heterogeneity in hematopoietic stem cell function. *Cell Stem Cell* 28:241–256e6. <https://doi.org/10.1016/j.stem.2020.09.018>
30. Cain TL, Derecka M, McKinney-Freeman S (2025) The role of the haematopoietic stem cell niche in development and ageing. *Nat Rev Mol Cell Biol* 26:32–50. <https://doi.org/10.1038/s41580-024-00770-8>