COMMENTARY

Testing the adaptive selection of human mtDNA haplogroups: an experimental bioenergetics approach

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The evolution of human mtDNA (mitochondrial DNA) has been characterized by the emergence of distinct haplogroups, which are associated with the major global ethnic groups and defined by the presence of specific mtDNA polymorphic variants. A recent analysis of complete mtDNA genome sequences has suggested that certain mtDNA haplogroups may have been positively selected as humans populated colder climates due to a decreased mitochondrial coupling efficiency, in turn leading to increased generation of heat instead of ATP synthesis by oxidative phosphorylation. If this is true, implying different evolutionary processes in different haplogroups, this could potentially void the usefulness of mtDNA as a genetic tool to study the timing of major events in evolutionary history. In this issue of the *Biochemical Journal*, Taku Amo and Martin Brand present experimental biochemical data to test this hypothesis. Measurements of the bioenergetic capacity of cybrid cells harbouring specific Arctic or tropical climate mtDNA haplogroups on a control nuclear background reveal no significant changes in coupling efficiency between the two groups, indicating that mtDNA remains a viable evolutionary tool to assess the timing of major events in the history of humans and other species.

Key words: bioenergetics, coupling efficiency, cybrid, haplogroup, mitochondrial DNA (mtDNA), mtDNA selection.

Mitochondria are ubiquitous organelles found in all nucleated cells and are the major generators of cellular ATP by OXPHOS (oxidative phosphorylation), a process dependent upon the coordinated expression and interaction of both nuclear DNA and mtDNA (mitochondrial DNA; i.e. the mitochondrial genome). mtDNA is a circular double-stranded DNA molecule of 16.6 kb in humans that encodes 13 essential polypeptides of the OXPHOS system and the necessary RNA machinery (two rRNAs and 22 tRNAs) for their translation within the organelle. mtDNA lacks introns and long repeat regions that are often associated with the nuclear genome, and is found in multiple copies within cells, the number of copies being dependent upon the cell type. It is the normal situation for these copies to be identical, a condition known as homoplasmy, but it has become apparent that on occasions more than one mtDNA species can be associated with an individual or cell, a condition known as heteroplasmy. Not surprisingly, given the role that the proteins encoded by the mitochondrial genome play in the production of ATP, mutations of mtDNA are an important and common cause of human disease, affecting approximately one out of every 3500 individuals [1].

The evolution of human mtDNA is characterized by the emergence of distinct lineages or haplogroups defined by specific mtDNA polymorphisms, with different haplogroups being associated with the major global ethnic groups [2]. The eight most ancient haplogroups, L0–L7, are specific to Africa [3]; two haplogroups derived from L3, M and N have populated the rest of the world. Haplogroup N encompasses haplogroup R, which includes all the major European haplogroups. The remainder of haplogroup N, and haplogroup M is made up of lineages found in Asian/Arctic populations.

mtDNA has been widely used to make inferences about the history of our and other species, and is a favoured tool of evolutionary biologists for several reasons. First, it has a high mutation rate calculated to be approximately 10 times that of the nuclear genome. This high mutation rate allows sufficient signal to be generated over short periods to make inferences about population history. Secondly, mtDNA inheritance in most species is clonal, transmitted solely down the maternal lineage without paternal contribution [4]. An exception to this rule was recently observed in humans [5]; however, there is no evidence that such exceptions have left a footprint on the mtDNA phylogeny of humans [4]. The effective clonality of mtDNA allows evolutionary histories to be assembled without the complexities introduced by bi-parental recombination. The utility of mtDNA as a tool for elucidating population history is also dependent on the presence of a molecular clock. The assumption that mutations 'click off' at a regular rate can be used to derive estimates for the timing of events within populations. All that is required for the assumption of a molecular clock to hold is that the same evolutionary process must be occurring in all lineages irrespective of the complexity of the process.

In addition to primary mtDNA disorders, it has been suggested that inherited mitochondrial variants may play a role in common diseases such as Alzheimer's disease and Parkinson's disease [6]. There have been many reports claiming that particular disorders are significantly associated with one mtDNA haplogroup, suggesting one or more haplogroup-associated polymorphisms modify risk to disease. The situation can be more complex, however, as the high mutation rate means that many changes occur at more than one place in the phylogeny (are associated with more than one haplogroup), making associations much more difficult to detect. The third possibility is that, in disease groups, there is a cumulative effect of multiple, phenotypically very subtle mtDNA mutations in a patient, and it is this combined effect that is the risk factor, something that is very difficult to test.

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There has also been a long-standing debate over the role of selection in mtDNA evolution. Human mtDNA shows striking regional variation, traditionally attributed to genetic drift [7]. The transmission of mtDNA mutations through families over many generations has also led investigators to believe that selection against deleterious changes may be weak [8]. However, for some years now there has been increasing evidence for purifying selection being an important factor in the evolution of mtDNA [9]. This begs the question as to what has been the most important factor in shaping the evolution of mtDNA: selection or demographics? Purifying selection, however, need not affect the utility of mtDNA as an evolutionary tool providing it was acting equally on all lineages. However, selection that acts differentially on lineages would 'shatter' the molecular clock model so widely used to make evolutionary inferences.

Recent work published by Doug Wallace's group at the University of California at Irvine [10,11] provided evidence for positive selection of inefficient mitochondrial haplogroups as an adaptation to cold climates, a finding which has implications for the use and continued use of mtDNA sequences in evolutionary studies. Wallace and colleagues analysed 104 complete mtDNA sequences from different climate zones classed as tropical (African haplogroups), temperate (European haplogroup) and Arctic zones (Asian haplogroups), studying the ratio of non-synonymous (Ka) versus synonymous (Ks) amino acid substitutions in the 13 mtDNA protein-coding genes in all possible pairs from the three groups. Their data showed that: (i) the *MTATP6* gene was highly variable in the mtDNAs from the Arctic zone (Asian haplogroups); (ii) *MTCYB* (encoding cytochrome *b* of complex III) was particularly variable in the temperate (European) haplogroups; and (iii) *MTCOI* (encoding cytochrome *c* oxidase subunit I) was notably more variable in the tropical (African) haplogroups. Based upon these analyses, they concluded that selection may have played a role in shaping human regional mtDNA variation and that one of the selective influences was climate. Perhaps the most striking finding was that the *MTATP6* gene product had the highest amino acid sequence variation of any human mtDNA gene, significant in that this encodes an important subunit of the ATP synthase complex. Subtle variations in function of this complex might lead to proton slip or leakage, affecting coupling efficiency of the organelle. Given that heat is produced when respiration is uncoupled from ATP synthesis, these authors proposed that inefficient haplogroups may have been selected to allow uncoupling and increased survival in colder climates.

Given the importance of these claims, this work has since been repeated by a number of other investigators using independent and larger data sets. The initial results were surprisingly not supported [12,13]. One such study included 784 additional mtDNA whole genome sequences plus 53 of the mtDNA sequences used in the original analysis, revealing marked differences between the European mtDNA haplogroup clusters (temperate climate), suggesting that gene-specific differences in the non-synonymous/synonymous rate ratio are produced between lineages even with the same geographic origin. Thus, unless it is assumed that the European mtDNA haplogroups evolved in geographic regions with different temperatures, the hypothesis presented by Mishmar et al. [10] and Ruiz-Pesini et al. [11] was either an artefact of the methodology or an incomplete explanation of the selective process.

In this issue of the *Biochemical Journal* [12], Taku Amo and Martin Brand describe a series of elegant bioenergetic studies which seek to specifically test the hypothesis that Arctic mtDNA haplogroups are associated with a lower mitochondrial coupling efficiency than tropical mtDNA haplogroups. In order to negate the influence of nuclear genetic effects, the authors have used a long-standing 'friend' of the mitochondrial geneticist, i.e. the *trans*-mitochondrial cytoplasmic hybrid or 'cybrid' cell, as their model system. Cybrids are generated by fusing donor cytoplasts (enucleated cells containing mitochondria) with an immortalized human cell line that has been completely depleted of endogenous mtDNA (mtDNA-less or ρ° cells). Given that ρ° cells have no functional respiratory chain and are dependent upon pyruvate and uridine supplementation for growth, loss of either of these two metabolic requirements can be used to select for transformants harbouring complementing (exogenous) mtDNA [14]. In the absence of animal models of mtDNA disease, cybrid cell technology has proved invaluable in many areas of mitochondrial biology, including determining the genetic origin of certain mitochondrial disorders, demonstrating the existence of heterologous mtDNA recombination in human cells as well as dissecting the functional and physiological consequences of pathogenic mtDNA mutations, and, in this paper, different mtDNA haplogroups [15].

The authors use a systems approach (modular kinetic analysis) to investigate the effects that different haplogroups have on the kinetics of selected components of the OXPHOS pathway in intact cybrid mitochondrial preparations, namely: (i) substrate (complex I- or complex II-linked) oxidation; (ii) proton leak across the inner mitochondrial membrane; and (iii) the phosphorylating system. A direct comparison between Asian mtDNA haplogroups (A, C, D; found in Arctic populations) and three African (L1, L2 and L3) mtDNA haplogroups did not reveal any significant differences in the kinetics of substrate oxidation between the two groups. Likewise, the rate of proton leak (the physiological mechanism by which protons can re-enter the matrix without being coupled to ATP synthesis, but implicit in heat generation) was not significantly different between the two groups, with Arctic mtDNA haplogroups surprisingly showing a lower rate of proton leak than tropical mtDNA haplogroups, contrary to what might have been predicted on the basis of the previous hypothesis [10,11]. Similarly, no differences were observed either in the rates of the phosphorylating system or, indeed, the respiratory control ratios between the two groups. Finally, calculations of the coupling efficiency, which would be predicted to be lower in the mitochondria bearing the Arctic mtDNA haplogroups and associated with increased heat production, showed that these were actually associated with better coupling than tropical mtDNA haplogroups, and not supportive of the hypothesis.

This is the first investigation to present experimental evidence into the hypothesis that inefficient mitochondrial haplogroups have been selected during the migration of modern humans as an adaptation to cold climates. It must be remembered, however, that the work presented in this paper was carried out on a single nuclear background (A549 lung carcinoma), and that the function of the mitochondrial respiratory chain is dependent on co-ordination and co-evolution of both nuclear and mitochondrial genomes. Accordingly, results may differ on a different nuclear background, such as the 143B osteosarcoma background, a point made by the authors themselves. Despite their frequent use as a mitochondrial genetic tool, cybrids are not the perfect system since these are derived from immortalized, tumour-derived cell lines that are known to be aneuploid and can exhibit marked differences in chromosomal make-up, even in uniform (clonal) cultures. In spite of these reservations, this experimental approach has, however, provided evidence to support the subsequent population-based analyses that indicate that climate has not been the driving force of the evolution of human mtDNA, and demonstrates how evolutionary studies can be complemented and enhanced by well-executed laboratory experiments.

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