Relationship of Mitochondrial DNA Haplogroups with Complex Diseases

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Abstract
Mitochondria are responsible for energy production in unicellular and multicellular eukaryotes. Apart from their major role in metabolism, mitochondria are involved in many other cellular processes. A new paradigm is needed to understand aging, aging-related illnesses, and complex diseases. Because of the long co-evolution of mitochondria with the cells, subtle variations in the function of these organelles could influence many organ systems. Understanding the extent of this influence will shed light on the pathophysiology of some of these diseases. This review will examine the history of mitochondrial DNA research, and explain the role mitochondrial DNA plays in relation to complex diseases and their outcomes, like metabolic diseases, cancer and obesity. Finally, we will explore some of the new paradigms used in this research.

Keywords
Mitochondrial DNA, Haplogroups, Obesity, Complex diseases, Association, Therapy response

Introduction
The mitochondrion is a special organelle because it is the main producer of cellular energy, has its own DNA (mtDNA), transcription and replication machinery [1]. The mitochondrial genome has substantial similarity to prokaryotic genomes. Therefore, it is believed that the mitochondrion is the remnant of an ancient symbiosis between a prokaryote and an eukaryote. First, the prokaryotic symbiont was incorporated into the eukaryotic cell as an organelle. Next, the majority of the prokaryotic genes (1500 approximately) moved into the nucleus (in human cells only 37 genes remain inside the mitochondrion). [2]. Since that time, mitochondria and cell have become so interdependent that nuclear genes involved in mitochondrial function must evolve more quickly to keep up with the mutation rate of mtDNA. This coevolution has been shown to be critical for optimal mitochondrial function and consequently affects human health [3].

More recently, the role of mitochondria in other cellular processes has been explored. For example, in the regulation of the cytoplasmic calcium level [4], apoptosis [5], and signal transduction [6]. Mitochondria also influence the human phenotype to a higher extent than previously assumed [7], since subtle variations in cell metabolism affect different organ systems in complex ways [8]. In addition, mitochondria play an important role in the aging process [9]. In this article we explore to what extent the analysis of mtDNA can help us understand certain complex diseases.

Early mtDNA Research
MtDNA possesses certain attributes that makes it ideal for specific applications. Early mtDNA research focused on retracing the steps our ancestors took when migrating out of Africa and into the other continents [10]. This research for example benefitted from the negligible recombination and faster mutation rate of mtDNA, which resulted in an increased resolution in the analysis of the ancestral lineages. Moreover, mtDNA has become a powerful tool in the forensic analysis of partially degraded samples because of its higher copy number in comparison to nuclear DNA [11].

This research also led to some discoveries in the field of medicine, when mutations on the coding mtDNA were found to be responsible for non-Mendelian hereditary diseases, which are known nowadays as mitochondrial diseases [12]. Efforts then focused on understanding how mutations in mtDNA arise, how they are selected in the cell population and how they are passed on to the offspring. Answers to these questions came from studying the phenomenon of mitochondrial heteroplasmy, which is partially responsible for the clinical variability observed in mitochondrial diseases [13]. Heteroplasmy occurs when mitochondrial genomes mutate during embryogenesis, which causes the formation of a variety of lineages that coexist in the same individual [14]. The intracellular accumulation of mutant mtDNA on this stage occurs by a poorly understood selection mechanism. Then, the asymmetrical distribution of mutated mitochondria during mitosis affects mitochondrial function in the daughter cells, and the
with the advances in sequencing technologies new research focused on searching associations between mtDNA and the so called “complex” diseases, known to involve multiple organ systems, and of elusive etiology [19]. Because mitochondria play a key role in metabolism, diseases like diabetes, metabolic syndrome and obesity were initially investigated. Later on, it was found that slight defects in the mitochondrial repair system could also help explain the etiology of neurodegenerative diseases like Alzheimer [23] and Parkinson’s disease [24]. Furthermore, mtDNA is explored in relation to cancer because of the role mitochondria play in the regulation of apoptosis [25].

Currently, mtDNA sequences can be classified according to the haplogroups they belong to. Members of a haplogroup have one or more distinct SNP’s in common, which they all inherited from a common ancestor. The haplogroups are named according to a letter of the alphabet, but their lexicographical order guards no relation to their genetic relationships. The most recent common ancestor of all mitochondria belongs to haplogroup L. This haplogroup gave rise to seven distinct haplogroups (L0–L6), which are currently located in Africa. The group of humans that migrated out of Africa belonged to haplogroup L3, consequently all non-African haplogroups come from this group alone. Haplogroup L3 gave rise to the M and N subclades. In particular, European haplogroups (I, J, K, T, U, V, W, H, U, and X) emerged from the N subclade. On the other hand, Asian haplogroups developed from both the N subclade (A, B, F, and Y) and the M subclade (C, D, G, Q, and Z). Finally, during the migration to the Americas, haplogroups C and D (from the M subclade) and haplogroups A, B and X (N subclade) became the predominant genotypes in this continent [26]. Thus, each haplogroup represents a major branching point in the evolutionary tree of the mitochondrial genome.

Haplogroups help distinguish populations that have evolved independently during recent human history [10]. Because the resolution of the haplogroups is limited by the mitochondrial rate of mutation, the SNPs located in the mitochondrial control region are especially useful because this region’s mutation rate is even faster than the rest of the mtDNA [27]. Consequently, if some diseases are affected by mitochondrial function, they should behave differently depending on the haplogroup of the patient.

In particular, haplogroup N9a was found to be significantly related to resistance to metabolic syndrome in Japanese women [28]. In southern Italy, haplogroup T was found to be a risk factor for morbid obesity [body mass index (BMI) >45kg/m²] [29]. In a Taiwanese population, haplogroup B4 was associated with diabetes [30]. And in a Caucasian population, haplogroup J increased the risk of age related macular degeneration [31] (Table 1).

Nowadays, high throughput DNA analysis tools, like microarrays and next generation sequencing are used to verify or reject the aforementioned associations. Additionally, new insights into the levels of heteroplasmy (which turned out to be higher than previously thought) [14], and the screening of larger sample sizes improved research quality and statistical power.

For example, full mtDNA sequences could be mined from an exome data set composed of 1000 individuals with type 2 diabetes and 1000 controls from a Danish population. Among the 2025 polymorphisms that were found to be associated to diabetes type 2, 393 had not been reported before [32]. A group in Germany reported no association between BMI and coding mtDNA using an array of 119 SNPs. But based on their results they recommended further analysis of control region variants [33]. Interestingly, another team found that some polymorphisms in the coding regions were associated with BMI, using a larger sample size [34].

The potential of these polymorphisms to be of any use in the treatment and understanding of these diseases remains to be seen. Currently, the main obstacle to mtDNA research is low sample sizes, because of high sequencing costs. Hopefully, better analysis will be

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**Figure 1: The mitochondrial efficiency hypothesis** proposes that mitochondrial lineages evolving in different environmental conditions will be shaped differently by natural selection, resulting in variations in their mitochondrial function. Those variations could explain the increased incidence of certain types of diseases. Using the mutation pattern they acquired during evolution, associations with such diseases may be made.

**The Relationship between mtDNA and Complex Diseases**

As mtDNA mutates more quickly than nuclear DNA [3], natural selection may shape the effect mitochondria have on metabolism and even on the functionality of organ systems on a shorter amount of time. For example, the mitochondrial efficiency hypothesis proposes that in warm climate adapted populations, the evolution of mtDNA favored the conversion of energy to adenosine triphosphate (ATP) rather than to heat. Such mitochondrial adaptations may be disadvantageous in modern western lifestyles characterized by physical inactivity and high calorie diets [20]. Thus, the mitochondrial efficiency hypothesis could help explain the increased susceptibility to diabetes observed in south Asian populations [21]. Likewise, in cold climate adapted populations, mtDNA favors heat production at the expense of efficient energy conversion, increasing the likelihood that the next mutation could severely compromise the cell’s energy production. This could explain why some populations like northern Europeans are more susceptible to mitochondrial diseases [22] (Figure 1).

This information led to the hypothesis that although dysfunctional mtDNA can affect more than one organ system subter mitochondrial variations may be more difficult to detect phenotypically. Therefore,
found mitochondrial mutations to be responsible for the modulation of clonal selection. Nevertheless, using a mathematical model, this mutation could become ubiquitous in the tumor cells because rapid tumor growth could be facilitated by an mtDNA mutation. For example, their mtDNA is bound to be plagued with difficulties. For example, to have a slower mutation rate than normal tissue [42].

recently shown to vary between epithelial and stromal breast cancer reprogramming [40]. Additionally, mitochondrial function was revealed how little we knew about the role of mitochondrial function in cancer. It is now known that the anaerobic metabolism in cancer revealed how little we knew about the role of mitochondrial function may affect the evolution of some diseases. For example, LOHN progression results in visual loss due to neuropathy more frequently in haplogroup J in carriers with the 11778 G to A or the 14484 T to C mutation [36]. Haplogroups have also been associated with outcomes in other non-mitochondrial diseases. Haplogroups H and R were found to be an independent predictor of survival in patients with severe sepsis [37,38]. Similarly, haplogroup T was found to be protective of lipoatrophy after highly active antiretroviral therapy [39]. Even though most of these findings require further validation, the potential role of mtDNA in the stratification of patients cannot be ignored.

One of the most controversial topics is the link between mtDNA and cancer. In 1920, the Nobel laureate Otto Heinrich Warburg hypothesized that tumorigenesis was the result of insufficient cellular respiration caused by mitochondrial damage. He postulated this after observing the anaerobic metabolism cancer cells possess even in the presence of oxygen (Warburg effect). Modern research has now revealed how little we knew about the role of mitochondrial function in cancer. It is now known that the anaerobic metabolism in cancer cells is not a result of mtDNA damage, but a well regulated metabolic reprogramming [40]. Additionally, mitochondrial function was recently shown to vary between epithelial and stromal breast cancer cells [41]. Moreover, a non-intuitive result revealed colon cancer cells to have a slower mutation rate than normal tissue [42].

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Clearly, because of the complexity of cancer cells, the analysis of their mtDNA is bound to be plagued with difficulties. For example, rapid tumor growth could be facilitated by an mtDNA mutation. This mutation could become ubiquitous in the tumor cells because of clonal selection. Nevertheless, using a mathematical model, one study reported that non-functional mutations could become established similarly during rapid tumor cell growth entirely by chance [43]. Despite the difficulties just mentioned, another group found mitochondrial mutations to be responsible for the modulation of metastatic potential in breast cancer cells [44], this was only accomplished by observing a decrease in metastatic potential after replacing the mitochondria inside the cancer cells with mitochondria from healthy cells.

Even though the analysis of mtDNA inside cancer cells is still rudimentary, cancer incidence and progression could still be associated to mitochondrial haplogroups in a similar fashion as with other diseases. Efforts into this area have found an increased predisposition for breast and esophageal cancer in Haplogroup N [45]. Also, haplogroup U was found to increase the risk of prostate and renal cancer in a North American population [46]. Another research team associated the North East Asian haplogroup CZD (Comprised of M8a, C, Z, D4 and D5) with good disease-free survival in oral squamous cell carcinoma patients [47].

The Relationship between mtDNA and Disease Outcomes

One of the first clinical uses of mtDNA was the identification of mutations responsible for mitochondrial diseases. Apart from helping in diagnosis, knowledge of the mutations did not improve treatment options significantly. Treatment for these diseases still relies heavily on symptomatic control, changes in diet and exercises regimens [35]. Nevertheless, data obtained so far about the frequency and location of the affecting mutations have provided some insights into these diseases. For example, LOHN progression results in visual loss due to neuropathy more frequently in haplogroup J in carriers with the 11778 G to A or the 14484 T to C mutation [36]. Haplogroups have also been associated with outcomes in other non-mitochondrial diseases. Haplogroups H and R were found to be an independent predictor of survival in patients with severe sepsis [37,38]. Similarly, haplogroup T was found to be protective of lipoatrophy after highly active antiretroviral therapy [39]. Even though most of these findings require further validation, the potential role of mtDNA in the stratification of patients cannot be ignored.

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Mitochondrial Function and Obesity

The World Health Organization defines obesity as the abnormal or excessive fat accumulation that may impair health. Clinically a BMI of over 30 defines obesity [53].

Nowadays, obesity has become an alarming health problem in the new century [54]. Current treatment for obese patients include recommendations for lifestyle changes, and diets which may be complemented with pharmacological treatment, performing bariatric surgery in difficult cases [55]. Weight loss often involves a serious commitment, sometimes with suboptimal results, and surely tests the level of trust and communication between patient and physician.

Moderate weight loss benefits the prognosis of patients with metabolic syndrome, insulin resistance, hypertension [56] and diabetes [57]. This is the main reason behind the understanding of obesity as a disease with future complications. Even so, a high BMI could be nothing more than a non-pathognomonic clinical indicator of disease, like fever in other diseases [58]. If it were found that obese patients responded in different ways to a standard weight loss strategy, the classification into treatment response groups would be justified and it would be possible to investigate the role the pathophysiology of obesity plays on these differences, giving additional options for prevention and treatment of these patients.

To our knowledge, the effect of diets on human mitochondrial function has not been studied so far. However, it has been reported that the liver mitochondria of mice maintained with calorie restriction acquired more cristae [59]. Another work found a change in mitochondrial number in orexigenic neurons from mice during the transition from fasted to fed state [60].

Following this line of inquiry, we are performing a pilot study to find associations between mtDNA and the response to calorie restriction diet in an overweight human population. We expect to discover polymorphisms that can predict the success of such diet which would be a useful biomarker in the stratification of obese patients undergoing treatment.

Conclusion

Mitochondria are unusual organelles with unorthodox genomics and a long history of co-evolution with eukaryotes. Subtle changes in their function could help explain the variability of complex diseases across populations and also the relationship with some neurodegenerative diseases that appear later in life. Mitochondria can be readily distinguished based on the analysis of mtDNA and such differentiation can be associated with functioning of the organism. Nevertheless, mtDNA research is still limited by lack of data and more time is needed to become useful as a biomarker.

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Conflicts of interest

We confirm that there are no known conflicts of interest associated with this publication and the financial support for this work has not influenced its outcome.

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