Evolution and disease converge in the mitochondrion

D. Mishmar*, I. Zhidkov

* Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
b National Institute of Biotechnology in the Negev (NIBN), Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

1. Introduction

The emergence of new species is a result of interplay between genetics and environment. Intuitively, the genetic material in the form of nucleic acids (DNA and/or RNA) should change in reaction to this interplay. However, concomitantly, the genetic material has to be well-protected from changes, since it encodes multiple factors that act in concert to ensure proper embryo development. Nevertheless, during the past ~3 billion years of life on earth, environmental conditions changed dramatically multiple times, and many organisms which were adapted to those ancient environments could no longer sustain life and reproduce. If those environmental changes were not accompanied by genetic adaptations, mass extinctions would eventually lead to the eradication of life. Since this obviously did not happen, and since mass extinctions indeed occurred many times during our planet’s history, it is imperative to assume that new life always emerged from the ashes like the legendary phoenix. The main difference from the legend is that the survival of life forms despite extinctions was most likely not due to magic, but because of the ability to adapt to changes. That, following Charles Darwin’s theory [1], lies in the basis of the very existence of genetic diversity. Such diversity provides a large enough repertoire of mutations to cope with multiple possible environmental conditions, and enable the survival of the fittest. Therefore, it is only reasonable that organisms that could survive environmental changes are those that either could cope with a wide variety of environments, or those that had the capacity to go through genetic alterations and adaptations. Indeed, the more specialized the organism, the harder it is to adapt to the changing environments. Therefore, environmental changes are likely to be associated with the appearance of diseases in complex organisms that did not harbor the capacity to allow well being in the new environmental conditions.

Although the above discussion constitutes a simplistic summary of the principles that govern the dynamic history of life on earth, one could logically find correlations between the appearance of many complex disorders in man today and ancient environmental changes during human evolution. It is therefore no wonder that the emergence of several infectious diseases correlates with the appearance of human sedentary life-style and the increase of human population density [2]. Likewise, the appearance of age-related diseases correlates with the two-fold extended life span in modern human populations from the mean age of ~35 years during the Paleolithic era [3] to over 70 today [4].

Unlike other complex disorders, age-related disorders such as Alzheimer’s and Parkinson’s diseases cannot be easily removed from the human population by natural selection, as their onset occurs many years after reproductive age. It may well be, that the genetic landscape of human populations during ancient times harbored genetic variants that allowed, and even played a role, in human survival in different environmental conditions, but today act as disease susceptibility factors. This is the logic that lies in the basis of the ‘common disease-
common variant’ approach to investigating the genetic basis of complex disorders [5]. An excellent example for this is the ‘thrifty genotype’ hypothesis explaining the high prevalence of metabolic disorders in modern times [6]. According to this hypothesis, during ancient times when food was less available, selection acted towards efficient energy-producing genotypes. When these genotypes encountered the prosperity and food availability of the western world today, they conferred increased tendency towards obesity and diabetes [6]. This led us to the hypothesis that the genetic changes underlying fundamental evolutionary processes, such as local adaptions, natural selection and the emergence of new species, follow a very similar scheme and obey the same rules as disease-causing mutations. These principles could be so similar, that the very same mutations could either cause disease or allow adaptation to certain environmental factors, such as in the classic case of sickle cell anemia and the resistance to malaria [7]. Moreover, while considering disease-causing mutations, it is clear that only a subset of the genetic alterations allow survival of the embryo to term, whereas the rest are negatively selected and are abolished via natural miscarriages. Similarly, mutations that comprise human genetic variation today constitute population-fixed (and hence common) variants that survived long term evolutionary processes, but also rare un-fixed variants that have yet to face natural selection.

In this perspective, we discuss the similarities rather than obvious differences between the genetic characteristics and evolutionary schemes of disease-causing mutations versus genetic variants in humans. We focus on a single genetic system that played central roles both in human evolution and in various complex disorders – the mitochondrial energy-producing system, oxidative phosphorylation (OXPHOS).

2. The unique mitochondrial genetic system

Unlike most eukaryotic cellular systems, mitochondrial functions are encoded by two genomes that notably differ in their mutation rates and their mode of inheritance. Whereas most of the ~1500 genes encoding mitochondrial functions are located in the nuclear genome (nDNA), 37 are encoded by the maternally inherited mammalian mitochondrial genome (mtDNA). Of these, seven encode subunits of NADH ubiquinone oxidoreductase (complex I, ND1–ND6, ND4L), one encodes a subunit of cytochrome bc1 oxidase (complex III, cyt b), three encode subunits of cytochrome c oxidase (complex IV, CO1–3), two encode subunits of F1–F0 ATP synthase (complex V, ATP6 and 8), 22 encode tRNAs and two rRNA genes (12S and 16S). As previously discussed, since animal mtDNA evolves at least an order of magnitude faster than the nDNA [8], tight co-evolution between nDNA and mtDNA-encoded genes should occur to maintain mitochondrial structure and function (Reviewed by [9,10]). Mutations occurring in the mtDNA are transferred to the next generation in a haploid manner, because of its maternal mode of inheritance. Inheritance of mtDNA mutations from generation to generation depends on the percentage of mutant mitochondria in the fertilized egg: at first, the mutated mtDNA is harbored by only a small portion of cellular mitochondria (heteroplasmy). In time, however, it could reach fixation in the germline, mostly via a severe bottleneck involving the random unbalanced partition of the cytoplasm during cell divisions (replicative segregation) in gamete formation, as well as over generations. Moreover, the severe bottleneck that reduces the effective population size of mitochondria occurs in the female precursor germ cells, resulting in the reduction of mitochondrial population from tens of thousands to ~200, thus improving the odds of low-prevalence mutations to reach fixation [11,12]. The apparently stochastic nature of this mutation fixation process could be altered when the mutation has an evolutionary advantage. Such is the case of large mtDNA deletions in somatic tissues, that on the one hand reduce essential gene content and cause disease, but on the other hand create smaller circular mtDNA which replicates faster than the full length mtDNA [13]. In addition, it has been suggested that the shift from heteroplasmy to homoplasmy of mtDNA mutations in nasopharyngeal oncocytic tumors could be due to selective advantages accompanying the oncocytic transformation [14]. The established pathological mtDNA mutations T8993C and T9176C, which cause Neuropathy, Ataxia and Retinitis Pigmentosa (NARP), conferred advantage in tumor growth in nude mice [15,16]. It is worth noting that it is not clear whether the increase of heteroplasmy level in these mutations over generations is due to some sort of selective advantage or just random genetic drift [17]. Taken together, as will be discussed below, these evidence support the hypothesis that both disease-causing mutations and mtDNA genetic variants respond to genetic drift as well as to negative or positive selection, thus underlining their functional similarity.

3. Mitochondrial–nuclear interactions in disease and evolution

Mitochondrial (mito)–nuclear genetic interactions were suggested to play a crucial role in adaptation as well as the creation of reproductive barriers [9,10,18]. In evolutionary time scale, multiple pieces of evidence exemplify that disruption of mito–nuclear interactions cause reduction in mitochondrial activity. Accordingly, nuclear transfer in cattle or cytoplasmic hybrid (cybrid) experiments in primates or rodents, which introduce the mtDNA from one species into cells harboring the nDNA from another species of the same genus [19–22], result in reduced mitochondrial activity. Similarly, altered mitochondrial activity was observed in cybrid experiments in humans, introducing mtDNAs into cells harboring the nuclear genome from different populations [23–25] as well as in backcross experiments in Drosophila, wasps, rats and the copepod Tigriopus californicus [26–29]. The mentioned backcross experiments also described reduced fitness in the inter-population hybrids. Hence the tight mito–nuclear co-evolution occurs not only at the species level, but could also lie in the basis of reduced fitness and hybrid breakdown in inter-population crosses [9,30–32]. It is therefore conceivable that mito–nuclear interactions could be important in maintaining mitochondrial structure and function within the human species. Indeed, pathological mutations causing Leber’s Hereditary Optic Neuropathy (LHON) [25], complex I-specific neurodegenerative disease [33] or Leigh syndrome [34] affect the assembly of OXPHOS complexes by interfering with the interaction between mtDNA and nDNA-encoded subunits. It is therefore conceivable that mito–nuclear interactions play a role not only in evolutionary processes but also in disease. Despite this similarity, it is yet to be determined whether disrupting the evolutionary scheme of co-evolving amino acid positions in interacting mtDNA and nuclear DNA-encoded factors cause disease.

4. Disease-causing mutations and genetic variants in the mtDNA, and their cross-talk with evolutionary forces

If the same evolutionary principles govern the dynamics of disease-causing mutations and common mtDNA genetic variants, several predictions emerge: (A) disease-causing mutations could be subjected to both negative and positive selection, (B) both disease-causing mutations and common genetic variants could respond to genetic drift, (C) mutations defining mtDNA genetic backgrounds play a role both in human response to different environmental conditions and in disease susceptibility, and (D) at least a subset of the mtDNA genetic variants should be functional. Indeed, all predictions are supported by evidence from real life.

More than a hundred disease-causing mutations have been described in the human mitochondrial genome [35]. Some of these mutations result in early onset whereas others cause late onset disorders, which are mostly systemic and thus affect many tissue types [35]. Of these mutations, most occur in highly conserved
nucleotide positions within the mtDNA coding region, consistent with their functionality [36]. These mutations can be insertions, deletions or point mutations, which cause diseases in different levels of heteroplasmy, i.e. deletions and insertions (generally speaking) could cause disease while inhabiting less than 60% of cellular mitochondria, whereas many point mutations will cause diseases only when their cellular level reaches around 100% [37]. Since disease-causing mutations could, by definition, reduce fitness, the apparent repertoire of disease-causing mutations is only a subset of the actual mutation rate. Indeed this logic applies to chromosomal aberrations, of which only a subset are found in fetuses whereas the vast majority are found in spontaneous abortions [38]. It is yet to be assessed whether the same logic applies to mtDNA mutations, namely that mtDNA disease-causing mutations represent those that are compatible with life and hence are only a subset of all generated mtDNA mutations.

Indeed, it is only reasonable that most disease-causing mutations are negatively selected, i.e. do not become fixed in the population. However, the action of adaptive selection on disease-causing mutations is far less trivial, as they have to confer some sort of evolutionary advantage. Although such an effect was not clearly demonstrated in people carrying mtDNA disease-causing mutations, there is evidence for differential functionality of mtDNA disease-causing mutations in different populations. Mutations causing Leber’s hereditary optic neuropathy (LHON) result in disease with varying severity depending on the mtDNA genetic background (haplotype, haplogroup) they occur on [39,40]. Specifically, certain LHON-causing mtDNA mutations may result in blindness, depending on whether they have occurred in combination with the mtDNA haplogroup J [41]. Moreover, the 14484 C8993T and C9176T grew exceedingly faster as compared to wild type expression of a mitochondrial disorder, but also could re

action of positive (adaptive) selection has been demonstrated in selection [36,47,48], and that mtDNA genetic variation played a role as mtDNA genetic variation was shaped by both negative and positive mtDNA deletions show altered phenotypic severity[43]. These data disease-causing mutations under certain conditions. A clearer positive action of selective forces in favor of the survival of some mtDNA positive selection at least at the cellular level. It is important to note, disease-causing mutations respond both to negative and possibly positive selection at least at the cellular level. It is important to note, that advantage conferred at the population level was not observed for mtDNA disease-causing mutations. In the case of mtDNA genetic variants, both negative and positive selection has been previously discussed [45]. Evidence for negative selection has been reflected in significantly reduced genetic variation within some mtDNA-encoded genes, and in the fact that most non-synonymous mtDNA variants are rare variants that still did not endure the effects of selection [46]. The action of positive (adaptive) selection has been demonstrated in human mtDNA variants [36,47,48]. Our rigorous analysis of whole mtDNAs from all major global populations has shown that human mtDNA genetic variation was shaped by both negative and positive selection [36,47,48], and that mtDNA genetic variation played a role as humans migrated from the warm climates of Africa to populate the considerably colder northern hemisphere. In summary, natural selection, certainly negative but possibly also positive selection, has left its mark in the pattern of global mtDNA genetic variation and possibly affected mtDNA disease-causing mutations.

It is widely accepted that population dynamics during the course of time has affected allele frequencies in different populations, largely due to the effect of genetic drift [49]. Interestingly, genetic drift also affects the prevalence of disease-causing mutations. Apparently most mtDNA disease-causing mutations are rare, segregate among families, and arise independently and repeatedly in combination with phylogenetically distant genetic backgrounds. These characteristics support strong negative selection against the fixation of these mutations. However, disease-causing mutations are also subjected to genetic drift. Although many of them first appear in a heteroplasmic state, they can eventually dominate the mitochondrial population in the cell (homoplasmic state) due to replicative segregation, namely due to the random division of the cytoplasm during cell division [17,50]. Such replicative segregation during cell division is frequently compared to the population-based genetic drift. Thus, the dynamics of both mtDNA disease-causing mutations and mtDNA genetic variants respond to random population dynamics and founder effects (Fig. 1).

Similarly to disease-causing mutations, multiple studies support the functionality of mtDNA genetic variants. Firstly, mtDNA genetic backgrounds (haplogroups) have been repeatedly associated with altered susceptibility to various complex phenotypes including Parkinson’s disease [51–56], type 2 diabetes and its complications [57–60], endurance athletics [61–63], various cardiovascular disorders [64–67], age-related macular degeneration [68–70], altered plasma lipid and cholesterol levels [71–73], schizophrenia [74,75], various types of cancer [76–78], human sperm motility [79–81], and successful aging [82–86]. It is worth noting that the high population divergence of mtDNA [87], and its close interplay with nDNA-encoded and environmental factors have resulted in the questioning of some of these associations [88–90]. Careful inspection of these studies reveals associations of certain haplogroups, such as haplogroup J and T in Europeans and haplogroup D in Asians, with multiple phenotypes, suggesting that these genetic backgrounds harbor functional mutations. Secondly, a comparison of cytoplasmic hybrids (cybrids) sharing the same nucleus but differing in their mtDNA genetic backgrounds showed differences in mitochondrial function, reflected by calcium uptake [23], the production of reactive oxygen species (ROS) [24] and the degree of resistance to apoptosis [91]. Thirdly, we have shown that a genetic variant defining haplogroup J in the mtDNA control region (C295T), which is not present in any other human lineage, altered the rate of in vitro transcription and consistently altered mtDNA copy number in cybrids carrying this variant as compared to cybrids with haplogroup H [92]. Therefore, common mtDNA variants and disease-causing mutations are not only subjected to similar selective forces at the population level, but both lead to functional consequences, explaining their appearance on the ‘radar screen’ of natural selection.

5. Recurrence of mtDNA ancient variants in the wrong genetic context could result in disease

So far we have demonstrated the similarity in the action of evolutionary forces on disease-causing mutations and mtDNA genetic variants. We also presented evidence from disease association studies and cell culture experiments supporting that at least a portion of haplogroup-defining mutations have functional properties. It follows that the recurrence of ancient variants as de novo mutations on the existing genetic background could be associated with disease. Indeed, as mentioned above, since nDNA and mtDNA-encoded factors tightly co-evolve, it is not surprising that the presence of mtDNA from a given species in the nuclear genetic background of another species results in altered mitochondrial function. However, what happens if mtDNA haplogroup-defining mutations occur as de novo mutations in the context of distantly related mtDNA genetic backgrounds? To test such a hypothesis, we chose to use tissue samples in which the mtDNA mutation rate is significantly increased, and many de novo mtDNA mutations accumulate. Such is the situation in various cancer types.
the similarity rather than the difference between disease-causing their survival in certain conditions. In this perspective, we underlined various genetic backgrounds or (B) due to natural selection favoring owing to the degree of their expressivity in combination with the genetic repertoire of their own. Some of these mutations will be more diversity, the mutations causing common diseases could form a means that of the similar mutations that comprise human genetic mutations in many other genes, each con.

One of the worries a clinical geneticist faces is the possibility that a disease-causing mutation found in a patient sample is con. Such a tendency was previously implied in meta-analysis of multiple tumors [93]. When counting recurrent de novo combinations of mutations, we noticed that they strikingly recapitulated major mtDNA haplogroups harboring combinations of up to 7 different mutations [97]. It is noteworthy that many mutational screens for mtDNA de novo mutations in several cancer types failed to identify any of the known mitochondrial disease-causing mutations, suggesting some type of negative selection against these alterations in cancer. This suggests that human evolution and the types of cancer that were studied are likely to be under similar selective constraints. Similarly, most mutations causing Familial Mediterranean Fever in the protein Pyrin recapitulate the primate ancestral state [98], thus further supporting the view that ancient variants could have functional consequences whenever their genetic context changes. These evidence imply that evolutionary principles not only govern the formation and occurrence of both disease-causing mutations and genetic variants, but that changing the genetic landscape could transform a relatively innocent genetic variant to a disease-causing mutation.

**6. Concluding remarks**

One of the worries a clinical geneticist faces is the possibility that a disease-causing mutation found in a patient sample is confined to his own family, and that the disease can be caused by many other mutations in many other genes, each confined to a certain family. This means that of the similar mutations that comprise human genetic diversity, the mutations causing common diseases could form a genetic repertoire of their own. Some of these mutations will be more common than others either (A) due to drift and population expansion owing to the degree of their expressivity in combination with the various genetic backgrounds or (B) due to natural selection favoring their survival in certain conditions. In this perspective, we underlined the similarity rather than the difference between disease-causing mutations and genetic variants, not only because both obey the principles and rules of evolution, but because in some conditions the very same mutations could be nearly harmless variants or cause disease. Thus, taking into account the principles of evolution while investigating the molecular basis of diseases could pave the path towards the discovery of the causes of diseases not necessarily in the form of novel mutations but also in the form of ‘Janus-like’ (two faced) genetic variants.

**Acknowledgements**

The authors wish to thank Naama Shani, BGu, for critical reading of the manuscript. This work was supported by grants from the Israel Science Foundation (ISF), Bikurah F.I.R.S.T. Foundation and Israel Cancer Association awarded to D.M. The authors also thank the Israeli Ministry of Absorption for a graduate student scholarship to IZ.

**References**


