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Consequences of Compromised Mitochondrial Genome Integrity

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Abstract

Maintenance and replication of the mitochondrial genome (mtDNA) is essential to mitochondrial function and eukaryotic energy production through the electron transport chain. mtDNA is replicated by a core set of proteins: Pol γ, Twinkle, and the single-stranded DNA binding protein. Fewer pathways exist for repair of mtDNA than nuclear DNA, and unrepaired damage to mtDNA may accumulate and lead to dysfunctional mitochondria. The mitochondrial genome is susceptible to damage by both endogenous and exogenous sources. Missense mutations to the nuclear genes encoding the core mtDNA replisome (*POLG*, *POLG2*, *TWNK*, and *SSBP1*) cause changes to the biochemical functions of their protein products. These protein variants can damage mtDNA and perturb oxidative phosphorylation. Ultimately, these mutations cause a diverse set of diseases that can affect virtually every system in the body. Here, we briefly review the mechanisms of mtDNA damage and the clinical consequences of disease variants of the core mtDNA replisome.

Keywords

POLG; *POLG2*; *SSBP1*; *TWNK*; Mitochondrial DNA; Mitochondrial disease; replication; mutagenesis

Introduction

An important and shared feature among all known multicellular eukaryotes is the presence of mitochondrial organelles. Mitochondria are home to the electron transport chain (ETC), through which oxidative phosphorylation generates over 90% of cellular ATP. To meet cellular demands, cells contain an interconnected network of sometimes thousands of mitochondria, and within each of these mitochondria are multiple copies of the mitochondrial genome (mtDNA). The mtDNA is found within the matrix of the mitochondrial network. Unlike the nuclear genome, the 16.6 kb mtDNA is organized into a compact circular molecule. As illustrated in Figure 1, mtDNA encodes genes for 13

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polypeptides that comprise essential subunits of the ETC complexes [1]. Furthermore, the mtDNA also encodes the 2 ribosomal RNA and 22 tRNA genes necessary for translation of the aforementioned 13 polypeptides (Figure 1).

Like nuclear DNA, mtDNA is susceptible to damage. Sources of damage to mtDNA include both exogenous and endogenous insults, such as environmental toxins, radiation, oxidation and intrinsic replication errors (Figure 2). In contrast to available repair pathways in the nucleus, repair of mtDNA damage is limited. Mitochondria do possess a robust base excision repair pathway (BER) involved in repair of oxidative damage and base deamination. However, mammalian mitochondria lack nucleotide excision repair [2], which, in the nucleus, functions to excise UV-induced crosslinks and bulky adducts. Mammalian mitochondria also lack ribonucleotide excision repair, mismatch repair [3], and end-joining pathways necessary for repairing double strand breaks (Figure 2). The absence of these DNA repair pathways in mammalian cells places mtDNA at greater risk for accumulation of unrepaired lesions, which can lead to fixation of mutations, large deletions, and roadblocks to replication and transcription. Mitochondria do possess additional mechanisms to cope with unrepaired DNA damage. mtDNA is pluriploid by nature, and replication of mtDNA is uncoupled from the cell cycle. Therefore, additional copies of mtDNA can be synthesized to assuage the impact of damage to individual genomes. If significant damage accumulates and the ETC is disrupted, mitochondria may fragment. This initiates a series of events to cleanse the mitochondrial pool through a mechanism known as mitophagy. During this process, fragmented mitochondria attempt to re-fuse. Those lacking a competent membrane potential will not fuse but instead are channeled for destruction by lysosomes [4].

Mitochondrial DNA replication is accomplished by a dedicated set of proteins all encoded in the nucleus. The genes *POLG* and *POLG2* encode the catalytic and dimeric accessory subunits of the DNA polymerase γ (Pol γ). The gene *TWNK* encodes the hexameric DNA helicase Twinkle, and *SSBP1* encodes tetrameric mitochondrial single-stranded DNA binding protein (mtSSB) [5–7] (Figure 3). The catalytic subunit of Pol γ contains both the DNA polymerase active site and a 3’ exonuclease proofreading site [8]. The accessory subunit functions to enhance DNA binding and increase processivity during DNA synthesis [9]. Abundant and growing evidence supports an asynchronous strand-displacement mechanism of mtDNA replication [10,11]. There is evidence in some cell types and tissues suggesting that alternate modes of replication, including strand-coupled replication and RITOL, may occur at lower frequencies [12].

Mutations to the nuclear *POLG*, *POLG2*, *TWNK*, and *SSBP1* genes have devastating repercussions for mitochondrial function and human health. The *POLG* gene is the most commonly mutated nuclear gene associated with mitochondrial disease [13]. At time of publication, there are over 300 known pathogenic mutations in *POLG* (http://tools.niehs.nih.gov/polg/). Dozens of additional mutations in the *POLG2*, *TWNK*, and *SSBP1* genes also result in human disease. As illustrated in Figure 3, disease variants of all four replisome proteins are associated with a number of changes to their biochemical functions [14–20]. These include changes to the rate and efficiency of replication fork progression and DNA synthesis, protein-DNA affinity, and protein and complex stability. Collectively, these altered functions lead to stalled replication forks, uncoupling of the

replication machinery, and aberrant exposure of vulnerable ssDNA. DNA breaks and stalled replication can lead to large DNA deletions, depletion of mtDNA, and an increase in point mutations.

The biochemical and molecular consequences of disease variants of mtDNA replication machinery cause complex and varied human disease (Figure 4). A hallmark of mtDNA replication diseases is multi-organ clinical manifestations, with a particularly high prevalence of adverse effects on the nervous system, eyes, and muscles. Mutations to the mtDNA replisome genes are reviewed in greater detail in [21]. The mitochondrial consequences of nuclear mutations are well-documented for *POLG* [14,22], *POLG2* [19], and *TWNK* [20]. Several disease-causing mutations in *SSBP1* were recently identified and are detailed in [15–18].

As the home of oxidative phosphorylation, mitochondria play a crucial role in ATP production, and healthy mtDNA is essential for human health. Multiple mtDNA diseases are caused by mutations of the nuclear genes encoding the core mtDNA replication machinery. Novel mutations in these genes continue to be identified, and a current challenge is understanding the link between these gene mutations and their associated phenotypes. Mutations in *POLG*, for example, can manifest as many different diseases and in a myriad of different body systems, and in some cases the same *POLG* mutation can produce dramatically different clinical presentations [14]. Furthermore, environmental factors may trigger or compound the progression of mtDNA replication diseases [23]. By continuing to study the connections between these gene mutations, the biochemical properties of the variant proteins they produce, and clinical manifestations observed in patients, we hope to better understand the variability in mtDNA replication diseases.

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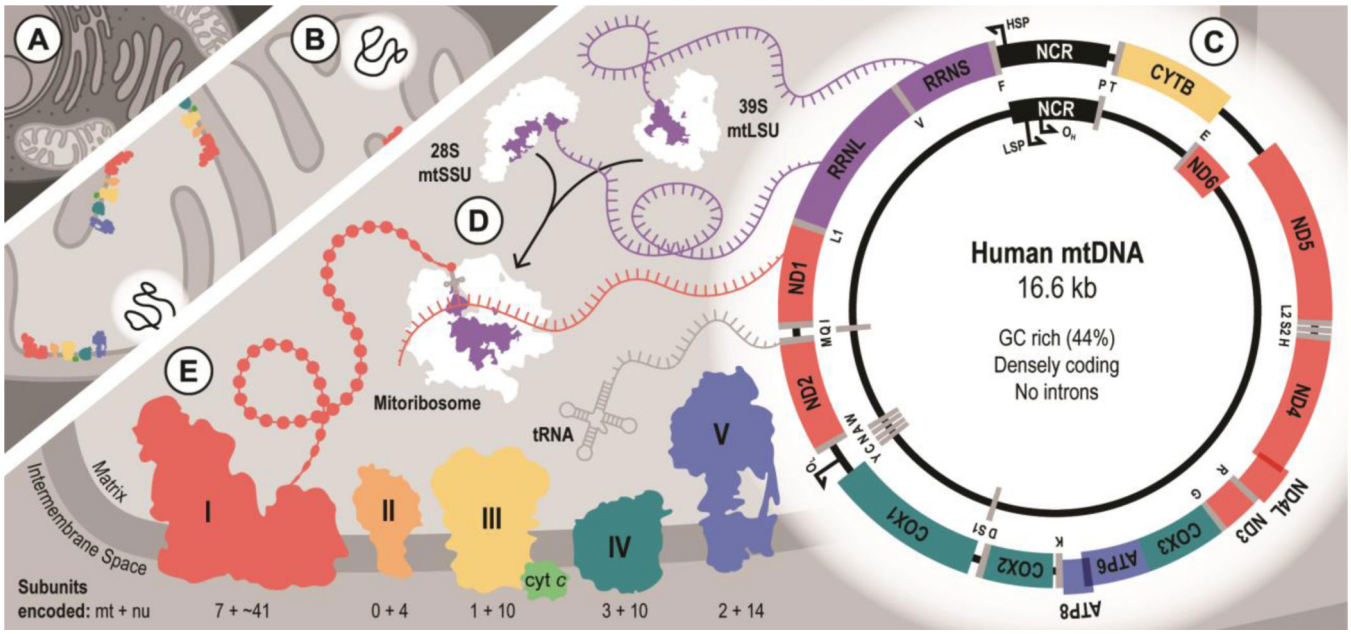

Figure 1. The mitochondrial genome encodes essential subunits of the electron transport chain (ETC).

Eukaryotic cells often contain multiple mitochondria (A), and within each mitochondrion are multiple copies of the mitochondrial genome (B). The human mitochondrial genome is a 16.6 kb circular molecule of dsDNA (C). The genome is compact and includes no introns. The only significant region that is not gene-coding is the non-coding region (NCR), which contains regulatory elements including the origin of H-strand replication (O_H) and transcriptional promoters for both the light and heavy strands (LSP and HSP). The L-strand replication origin (O_L) resides outside the NCR, between the genes *COX1* and *TRNW*. The H-strand of mtDNA is G/T rich while the L-strand is C/A rich; the genome overall is G/C rich relative to the human nuclear genome. The mtDNA encodes two rRNAs (purple) and 22 tRNAs (grey). Along with nuclear-encoded ribosomal proteins, these RNAs provide the core machinery necessary to translate the 13 polypeptides encoded in mtDNA (D). These polypeptides are then incorporated as subunits of the complexes of the ETC (E). mtDNA genes are color coded to match the ETC complexes containing their protein products. ETC Complex I (red) includes 6 subunits encoded in mtDNA, Complex III (yellow) includes 1, Complex IV (dark green) includes 3, and Complex V (blue) includes 2 [1]. Complex II (orange) is entirely encoded in the nucleus. Note that artistic license has been taken for this figure in the interest of simple illustration of RNA transcripts; in reality, the mitochondrial genome is transcribed as polycistronic transcripts which are then processed into mature RNA species [24].

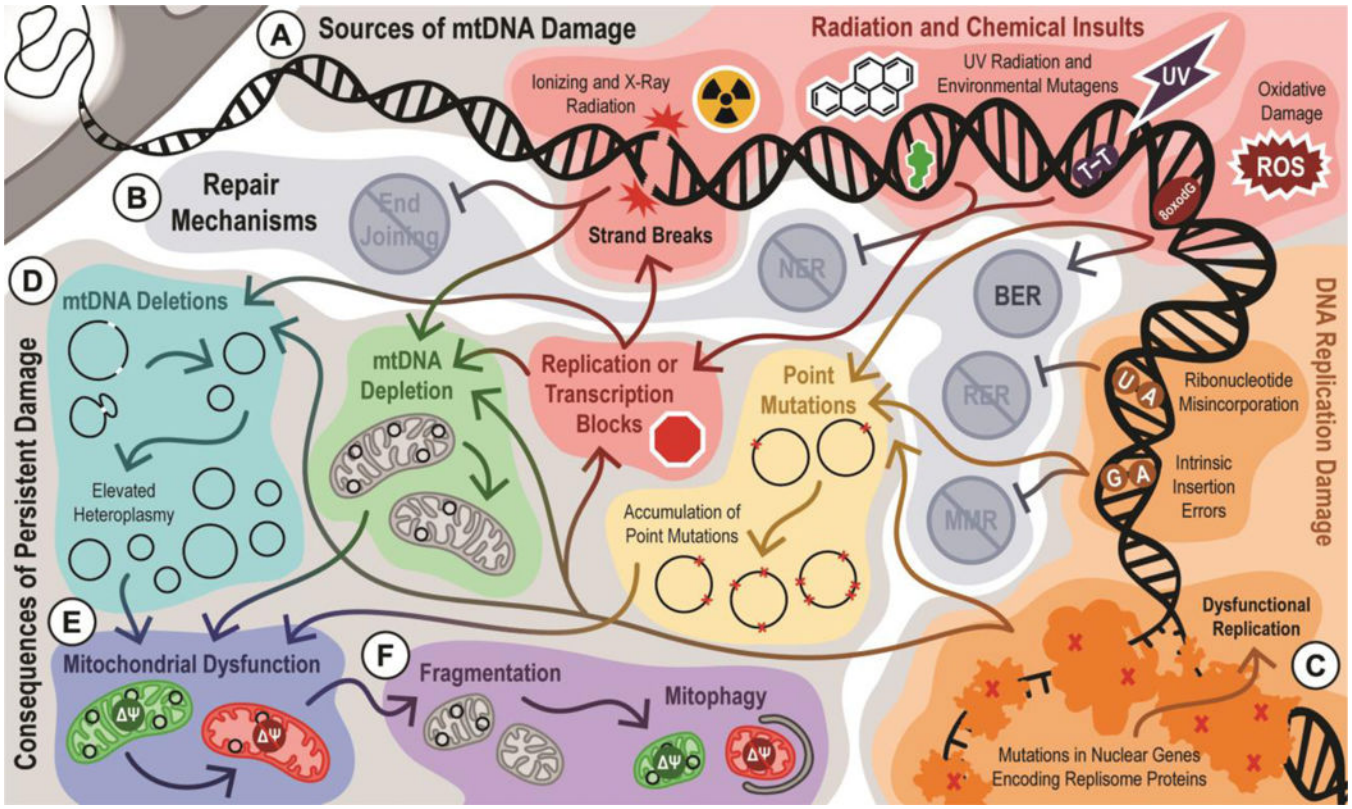

Figure 2. Accumulation of unrepaired mtDNA damage results in mitochondrial dysfunction.

mtDNA is susceptible to damage from both exogenous and endogenous sources (A). Oxidative damage to mtDNA is generally repaired through a BER pathway. Mitochondria lack mismatch repair (MMR), ribonucleotide excision repair (RER), end-joining, or nucleotide excision repair (NER) pathways [2,3]. For this reason, intrinsic replication errors not corrected by the Pol γ exonuclease, misincorporated ribonucleotides, and damage from radiation and environmental toxins are not repaired (B). Disease variants of the nuclear-encoded mtDNA replication machinery can also introduce damage (C). Damaged mtDNA has several fates (D). In the absence of repair pathways, mtDNA may accumulate point mutations and deletions. Furthermore, the pool of mtDNA may shrink as a result of damage or blocks to replication, yielding mtDNA depletion. Collectively, these outcomes may lead to mitochondrial dysfunction and loss of membrane potential (ΔΨ) [4] (E). Dysfunctional mitochondria may be cleared through mitophagy (F). Combined with enhanced mitochondrial biogenesis and replication of undamaged mtDNA molecules, mitophagy helps to recover mitochondrial function. However, recovery may not be possible in cases of extreme depletion or damage.

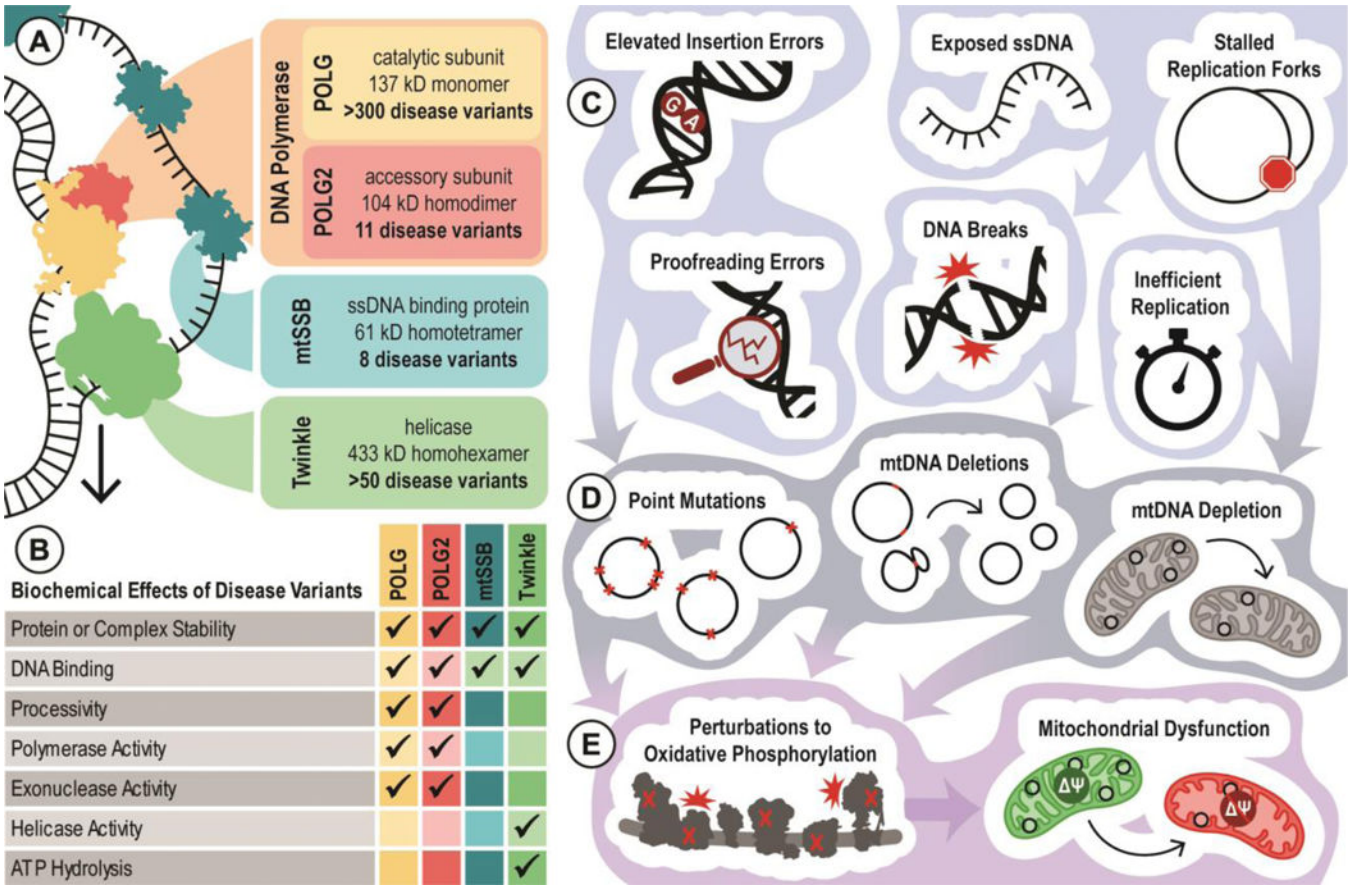

Figure 3. Disease variants of the mtDNA replication proteins lead to mtDNA damage and perturbations in oxidative phosphorylation.

mtDNA is replicated by a core set of nuclearencoded proteins [6] (A). Shown in yellow and red is the heterotrimeric DNA polymerase γ. It is composed of the large catalytic subunit POLG (yellow) and the dimeric accessory subunit POLG2 (red) and carries out both synthesis and proofreading functions. POLG2 contributes to the processivity of the polymerase. The tetrameric single stranded DNA binding protein mtSSB (teal) partially coats and protects exposed ssDNA during replication, while the hexameric DNA helicase Twinkle (green) unwinds dsDNA ahead of the polymerase. The replisome is shown displacing the H-strand of DNA as a nascent H-strand is synthesized. The displaced H-strand is bound by mtSSB. Human disease alleles in the genes encoding the four core replication proteins – *POLG*, *POLG2*, *SSBP1*, and *TWNK* – have been identified [14–20,25]. Disease variants of the replication proteins possess altered biochemical functions (B). These changes rarely result in gross disruption of function, instead often yielding subtle changes in enzyme activity, DNA affinity, or protein or complex stability. These problems can lead to increased error rates during DNA synthesis, decoupling of the replication fork, exposure of vulnerable ssDNA, and inefficient replication, among others (C). In turn, replication stress caused by dysfunctional replication proteins results in mtDNA depletion, deletions, and point mutations (D). Severe mtDNA depletion or the accumulation of damage causes perturbations in the ETC, disturbing mitochondrial homeostasis, reducing the membrane potential, and leading to mitochondrial dysfunction (E).

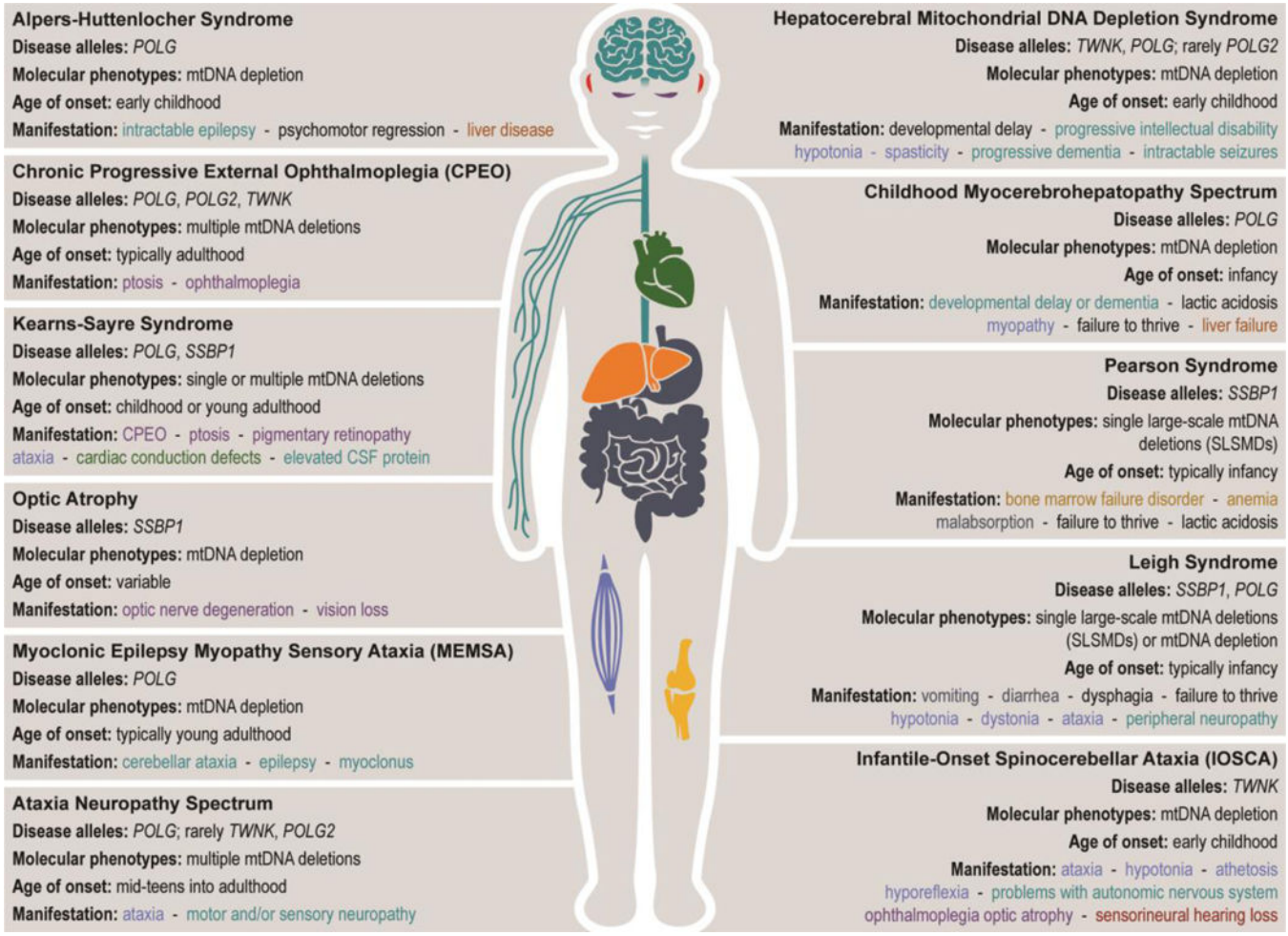

Figure 4. Mutations in nuclear-encoded mtDNA replisome genes cause complex and diverse human diseases.

mtDNA replication diseases manifest across many organ systems, although the eyes, nervous system, and muscles are most frequently affected. Shown here are the most common clinical manifestations of the 11 syndromes and disease spectra associated with mutations in the *POLG*, *POLG2*, *SSBP1*, and *TWNK* genes [14–22]. These diseases are characterized by mtDNA depletion or deletions. Notably, not all manifestations are observed in all patients, and there is considerable overlap of symptoms across the diseases arising from these mutations.