RESEARCH PAPER

CARDIAC AGING AND MITOCHONDRIAL DYSFUNCTION: IMPLICATIONS FOR CARDIOVASCULAR DISEASE

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Abstract
Cardiac aging is a major risk factor for cardiovascular diseases (CVD), and understanding its underlying mechanisms is crucial for developing effective anti-aging treatments. This review paper provides a comprehensive overview of the molecular processes underlying cardiac aging, with a focus on mitochondrial dysfunction and its role in CVD. We examine the contributions of various pre-clinical models, including dog, fruit fly, and mice, to our understanding of cardiac aging processes. The review also explores the intricate interplay between mitochondrial dysfunction, oxidative stress, and age-related changes in cardiovascular tissues. We discuss the potential of mitochondrial DNA copy number, energy generation, calcium regulation, and DNA methylation as diagnostic and therapeutic targets in metabolic and cardiovascular disorders. The review also highlights the complex relationship between mitochondrial dysfunction and CVD, including the role of oxidative stress, DNA mutations, and impaired oxidative phosphorylation. Ultimately, this review aims to provide a comprehensive understanding of the molecular processes underlying cardiac aging and the pivotal role of mitochondrial dysfunction in the pathogenesis of CVD.
Keywords: Cardiac aging, cardiovascular disease, mitochondrial dysfunction, mitochondrial DNA, oxidative stress, mitochondrial dynamics, mitochondrial DNA methylation, mtDNA copy number, energy metabolism, calcium regulation.
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Introduction

Cardiovascular (CV) disorders cause approximately one third of global mortality. One of the major CV risks is ageing. Cardiac ageing is a progressive deterioration of heart structure and function that occurs with age. Left ventricular hypertrophy and diastolic function impairment frequently develop in older individuals. As people age, calcific aortic valve disease and cardiac fibrosis contribute to the progression of aortic valve stenosis, while changes in the ventricles and valves increase the heart's susceptibility to stress and raise mortality rates from cardiovascular disease. In older individuals, heart displays reduced amount of muscle cells, higher size of cardiac muscle cells, and enhanced deposition of fats and fibrosis. he interdependence of the core processes of heart ageing, along with the relationships between cellular and molecular ageing and disorder-specific pathways, is quite complex [1,2]. Investigating the processes underlying heart aging may lead to the discovery of anti-aging treatments that protect against cardiovascular disease (CVD) [3].

In order to shed light on possible targets of cardiac ageing, it is necessary to gain information on appropriate pre-clinical models that may be applied to research the heart ageing processes. Studies using dog models have shown the progression of hypertrophic cardiomyopathy, as well as the deposition of amyloid and lipofuscin, which increase heart muscle stiffness. Since the canine heart conduction system and electrophysiology and human cardiac characteristics have a lot in common, canine model was largely applied for electrophysiology studies [4]. The hearts of fruit flies also exhibit similar properties, such as susceptibility to age-related functional impairments. Several genes in mammals that are responsible for oxidative stress (OS) and hypertrophic cardiomyopathy also affect heart ageing in Drosophila melanogaster [5,6]. Older rhesus macaques show mitral annular calcification and aortic valve calcification , hypertrophic cardiomyopathy, deposition of lipofuscin, fibrosis of interstice, myocardial infarction, and congestive heart failure [7,8]. Hearts of older mice demonstrated higher fibrosis, accumulation of amyloid, and elevated muscle cells size, as well as systolic and diastolic dysfunction. Hearts of older rats show hypertrophied cardiac muscle cells, high fibrosis and systolic and diastolic dysfunction [9,10].

The precise processes underlying heart ageing are yet to be explored. Available data show that heart ageing is associated with organelle function impairment. Deterioration of mitochondria (Mt) functioning in elderly individuals is believed to be the basis of senescence physiology. Study on Mt, which is considered a powerhouse of a cell and is responsible for providing energy for the heart, are not limited to bioenergy [11]. Recent research results indicate that mitochondria (Mt) also serve as metabolic transit sites and signaling hubs within cells, mediating cellular

function. Cardiac subsarcolemmal (SSM) and interfibrillar (IFM) mitochondrial subpopulations share common structure but Mt cardiac populations differ biologically. However, Mt dysfunction in senescence was confined to population of interfibrillar Mt [12,13].

Mt-induced OS is crucial for heart senescence processes as it is involved in non-convertible Mt DNA injury. electron transport chain (ETC) enzymes are located on the mitochondrial inner membrane (MIM) enclosing the Mt matrix which comprises Mt DNA. Elevated Mt reactive oxygen species (ROS) may injure DNA, which destabilizes appropriate provision of adenosine triphosphate in heart senescence, impairs the apoptotic processes, Mt biogenesis (MB) and bioenergetics [14,15].

MB is a process responsible for the amount of Mt. Mt functioning highly relies on the Mt morphology. Altered amount, size or location of Mt can lead to considerable change in functioning. Mt participates in such processes as fission, fusion and moving, that are called Mt dynamics. Mt morphology is essential for developing, cellular death, apoptotic mechanisms. The controlled release of mitochondrial genes and nuclear-encoded mitochondrial proteins (NEMPs) is part of MB. In this context, we emphasize specific mitochondrial DNA related to MB, oxidative metabolic processes, and the potential clinical applications of mitochondrial DNA in cardiac senescence [16]. We summarized key mitochondria-related aspects of cardiac aging and cardiovascular disease in Figure 1.

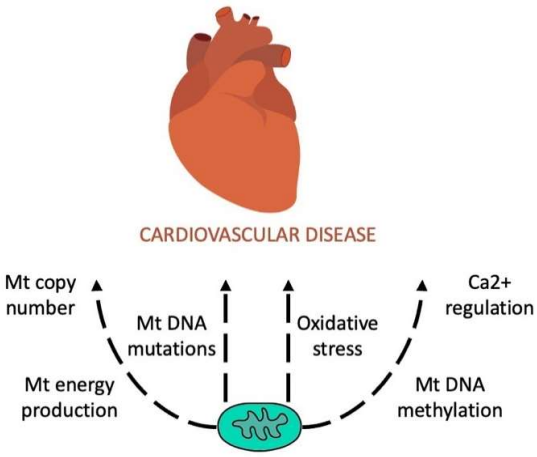

Figure 1. Key aspects of the role of mitochondria in the CVD development.

Methods

To comprehensively review the molecular processes underlying cardiac aging, with a specific focus on mitochondrial dysfunction and its association with cardiovascular diseases (CVD), we conducted a systematic literature search. This search aimed to gather relevant studies published up to October 2023, employing a structured strategy to optimize inclusivity and relevance while upholding high standards of rigor.

The literature search was performed across several electronic databases, including PubMed, Web of Science, Scopus, Google Scholar, and the Cochrane Library. These databases were selected for their extensive collections of peer-reviewed biomedical literature, interdisciplinary research, and systematic reviews related to our area of interest.

We established clear inclusion and exclusion criteria to guide our selection process. Studies were included if they focused on cardiac aging, mitochondrial dysfunction, and cardiovascular diseases, encompassing both preclinical and clinical investigations. Relevant articles published in English were considered, particularly those examining specific molecular mechanisms such as mitochondrial function, oxidative stress, mitochondrial DNA (mtDNA) mutations, mtDNA copy number, and calcium regulation. Conversely, we excluded articles that primarily addressed non-cardiovascular diseases unless they directly related to cross-relevant molecular mechanisms. Additionally, review articles and meta-analyses without original data, studies unrelated to the defined molecular processes, and duplicates were not considered.

The selection process unfolded in a systematic manner, comprising several key steps. Initially, we screened the titles and abstracts of all retrieved records for relevance, removing any duplicates. Subsequently, the studies identified during this initial screening were evaluated in full to confirm their compliance with the inclusion criteria. Two independent reviewers conducted this assessment, and any discrepancies were discussed to reach consensus; a third reviewer contributed to the final decisions when needed. Following this, a standardized data extraction form was employed to capture essential data points from each selected study, including authorship, publication year, study type (e.g., in vivo, in vitro, clinical trials), population or model organism details, key investigated molecular processes, and main outcomes.

Data were analyzed qualitatively to identify key themes that emerged regarding the interrelationships among mitochondrial dysfunction, oxidative stress, and cardiovascular aging. This synthesis of findings facilitated thematic discussions on potential molecular targets for therapeutic intervention, thereby enhancing the overall transparency and reproducibility of our methodology.

By employing this systematic approach combined with rigorous selection criteria, we aimed to ensure the credibility of our review, ultimately providing critical insights into the molecular dynamics of cardiac aging and their implications for therapeutic development in cardiovascular diseases.

Mt genome organization, functioning and dynamics

Circular double-stranded mitochondrial DNA (ds-Mt-DNA) is approximately 16,500 nucleotides long and constitutes the mitochondrial genome. The mitochondrial genome encodes thirty-seven genes, including thirteen structural genes, twenty-two transport RNAs, and two ribosomal RNAs. The

nuclear genome encodes the remaining proteins necessary for mitochondrial function, transcription, repair, and maintenance, which are then transported to the mitochondria [17,18]. Because each organelle contains a different number of mitochondrial DNA copies, changes can be either homo- or heteroplasmic; that is, all copies may contain the same mutation, or different copies may possess different mutations. Mutated mitochondrial DNA is often associated with various disorders. These mutations can be heritable, acquired, or deposited. Mt mutations impact relies on the afflicted tissue and extent of heteroplasmy. Phenotype becomes mutation-specific if some particular extent of heteroplasmy has been exceeded [19,20]. Since mitochondrial mutations are typically masked by wild-type (WT) copies, this extent is usually above 70 percent. However, this number may vary by tissue type [21].

The generation of energy through the production of adenosine triphosphate is the primary function of mitochondria. Production of adenosine triphosphate has four major steps. First, pyruvate and fatty acids (FAs) are converted to acetyl coenzyme A. Second, tricarboxylic acid cycle uses acetyl coenzyme A to synthesize NAD + hydrogen. Third, transport of electrons from NAD + hydrogen to oxygen through ETC. Fourth, adenosine triphosphate is produced by membrane adenosine triphosphate synthase [22]. Genesis of adenosine triphosphate occurs simultaneously with genesis of reactive oxygen species, that takes part in regulation and signaling. However, excessive production of reactive oxygen species can lead to oxidative damage to mitochondrial DNA, fats, and proteins. Presently, overproduction of reactive oxygen species and consequent injury are believed to be essential parts of atherosclerosis development [23,24].

Mitochondria control calcium homeostasis by interacting with the endoplasmic reticulum, which stores calcium in the cell. The processes of calcium influx and efflux are critical for hormone and neurotransmitter release, cell signaling, regulation of mitochondrial membrane potential, and respiration. Moreover, Mt lipid metabolic disorders can trigger endoplasmic reticulum stress and unfolded protein response through Mt-endoplasmic reticulum contact spots [25,26].

Mechanisms of mitochondrial biogenesis, turnover, and recycling focus on the even distribution of mitochondria among cells, the maintenance of undamaged and functional mitochondria, and the repair of injured mitochondria. Mt turnover involves fission and fusion (division and merger) of injured organelles. Divided damaged parts of Mt undergo degradation through Mt autophagy. Undamaged parts of Mt undergo fusion and keep acting normally in the Mt network [27,28].

Mt fission mostly relies on DNM-1L and FIS-1 genes, and fusion relies on MFN-1, MFN-2 and OPA-1. Release of these proteins and functioning of the related proteins are highly controlled. Overproduction or abnormal activity of these proteins can disrupt mitochondrial autophagy, which is linked to various human disorders, including cardiovascular disease. However, the essential role of mitochondrial turnover suggests that these processes could serve as promising therapeutic targets. In a recent study in DRP-$1^{+/-}$ murine model of abdominal aortic aneurysm, Mt division inhibitor 1 helped to demonstrate the importance of Mt fission in progression of abdominal aortic aneurysm [29,30].

Mutated nuclear genes encoding respiratory chain (RC) and proteins needed for Mt DNA sustenance, genesis, fission-fusion, can further destabilize Mt genome and function of Mt. Mutations in OPA-1, SLC25A-4, and DRP-1 are associated with cardiomyopathy and mitochondrial fragmentation. Likewise, cardiomyopathy was identified due to nuclear genes needed for oxidative phosphorylation, such as complex 1, 3, 4 and 5 assembly factors (e.g., NDUFAF1, ACAD9, UQCC3, COA5, TMEM70) [31,32].

Multiple studies are presently concentrated on Mt disorders. While many details remain unclear, several novel therapeutic approaches have been developed. Several powerful strategies may be identified: increase effectiveness of ETC with the use of thiamin, ubiquinone, idebenone; ameliorate energy buffer with the use of creatine, protect CL with the use of elamipretide, improve synthesis of nitric oxide with the use of citrulline and arginine, and improve anti-oxidant supply with the use of vitamin C, D, E. Moreover, transplant of Mt, Mt gene treatment and use of pharmacology to control Mt metabolic processes are presently being closely studied [33,34].

Molecular processes underlying Mt DNA mutations

The precise processes linking mitochondrial DNA mutations to various cardiovascular diseases have yet to be elucidated. Numerous trials suggest that mitochondrial DNA mutations may impair mitochondrial homeostasis, leading to the overproduction of reactive oxygen species, disruptions in calcium metabolism, and a decrease in energy generation [35,36].

Impaired oxidative phosphorylation in mitochondria can disrupt energy metabolic processes and aberrantly alter the Na+-Ca2+ exchange, resulting in excessive Ca2+ accumulation in the cytosol, impaired diastolic function of cardiac muscle cells and smooth muscle cells (SMCs), and hypertension [37,38].

Mutations in transfer RNA genes

In Table 1, we summarized the range of point mutations and their effects on mitochondrial function. Transfer RNA genes are typically highly conserved due to their essential role in mitochondrial protein synthesis. Thus, mutations in transfer RNA can destabilize its structure, potentially leading to changes in its secondary structure. This effect has been observed for the mitochondrial mutation 10398 A > G in the MTND3 gene, which is associated with kidney disorders related to hypertension [39,40].

Cytoplasmic hybrids carrying the mutation 15910 C > T exhibited a 37.5% reduction in transfer RNA^{Thr} compared to controls, suggesting that this mutation may accelerate its degradation. Earlier, the same results were reported for different transfer RNA mutations: Mt 3253 T > C transfer $RNA^{Leu(UUR)}$, Mt 3243 A > G transfer $RNA^{Leu(UUR)}$, Mt 10003 T > C transfer RNA^{Gly}, Mt 5655 T > C transfer RNA^{Ala}, Mt 7551 A > G transfer RNA^{Asp} and Mt 14692 A > G transfer RNA^{Glu} [41,42]. Consequently, changes in transfer RNA levels result in decreased mitochondrial protein synthesis and lower mitochondrial protein concentrations in cells with mutations, as well as altered activity of complexes I and III, increased electron release, and enhanced generation of reactive oxygen species. Increased reactive oxygen species generation can lead to injury to cellular components and phenotypes associated with various disorders [41,42].

While there is a strong possibility that additional factors, including genomic and environmental influences, contribute to the progression of cardiovascular diseases beyond mitochondrial mutations, such as genomic and environmental factors. For example, methylation of mitochondrial DNA in platelets has been identified as a novel marker for predicting cardiovascular disease. Reports have indicated that methylation of mitochondrial DNA may be impaired by air pollution and other environmental factors, leading to disrupted mitochondrial function and subsequently impairing cardiac activity [43]. Similarly, mitochondrial DNA methylation can be influenced by the intake of levocarnitine and TMAO supplements, which have been established as markers of cardiovascular disease. While the precise processes underlying mitochondrial DNA methylation and its effects have yet to be clarified, elevated levels of trimethylamine N-oxide have been associated with an adverse lipid profile and an increased risk of cardiovascular and cerebrovascular diseases Antioxidant supplements can also modify mitochondrial DNA methylation patterns and lead to positive changes related to type 2 diabetes, Alzheimer's disease, malignancy, and atherosclerosis [44].

Table 1. Transfer RNA Gene Mutations and Their Effects on Mitochondrial Function

Mutation	Gene	Effect on tRNA	Impact on Mitochondrial Function
Mt 10398 A > G	MTND3	Destabilization of tRNA	Reduced mitochondrial protein synthesis; increased ROS
Mt 15910 C > T	-	37.5% less tRNA^Thr compared to controls	Increased degradation of tRNA, decreased protein genesis
Mt 3253 T > C	transfer RNA^Leu(UUR)	Change in tRNA structure	Decreased mitochondrial protein concentrations; altered complex 1 and 3 activity
Mt 3243 A > G	transfer RNA^Leu(UUR)	Change in tRNA structure	Decreased mitochondrial protein concentrations; altered complex 1 and 3 activity
Mt 10003 T > C	transfer RNA^Gly	Change in tRNA structure	Decreased mitochondrial protein concentrations; altered complex 1 and 3 activity
Mt 5655 T > C	transfer RNA^Ala	Change in tRNA structure	Decreased mitochondrial protein concentrations; altered complex 1 and 3 activity
Mt 7551 A > G	transfer RNA^Asp	Change in tRNA structure	Decreased mitochondrial protein concentrations; altered complex 1 and 3 activity
Mt 14692 A > G	transfer RNA^Glu	Change in tRNA structure	Decreased mitochondrial protein concentrations; altered complex 1 and 3 activity

Mt oxidative stress and Mt DNA mutation in heart senescence

Available data indicates that during heart senescence, mtDNA is exposed to high levels of OS. Because of histone deficits, limited DNA repair capabilities, and the proximity of mtDNA to sites of mitochondrial ROS synthesis, mtDNA is vulnerable to various types of damage, including point mutations, deletions, and a reduced number of mtDNA copies. Oxidative stress can damage mtDNA in various ways, including single- and double-stranded breaks as well as the formation of oxidized bases, such as 8-oxo-guanine (8-oxo-Gua) [45]. Prolonged replication of mtDNA and its nucleoid structure render mtDNA susceptible to oxidative stress and mutations. TFAM, POLRMT, SSB, twinkle protein and DNA Pol-gamma are the main nucleoid-related Mt proteins. DNA Pol-gamma and TFAM are essential for metabolic processes. Excessive expression or deletion of these proteins has been reported to contribute to the progression of cardiac dysfunction in transgenic murine models [46]. Homozygosity for mutations in mtPOLG ($POLG^{m/m}$) in murine models leads to hypertrophic cardiomyopathy, accelerates senescence, and results in the accumulation of mtDNA deletions and mutations. 8-oxo-Gua is a common product of mtDNA oxidation and is considered a cellular biomarker of oxidative stress-related DNA damage. Earlier in vitro trials demonstrated that mitochondrial transcription factor A (mtTFA) primarily binds to 8-oxo-Gua, which hampers the DNA repair process. The clearance of 8-oxo-Gua involves a complex mechanism that relies on proteins encoded by the MUTYH and OGG1 genes. MUTYH removes adenine that is incorrectly inserted opposite 8-oxo-Gua. OGG1, on the other hand, removes 8-oxo-Gua from an adenine mispair in human mtDNA by catalyzing the cleavage of the N-glycosidic bond between the damaged 8-oxo-Gua base and deoxyribose [47,48]. OGG-1 is an important enzyme for base excision repair of 8-oxo-Gua lesions. DNA polymerase gamma (Pol gamma) is essential for mtDNA replication and is associated with both Twinkle protein and single-stranded DNA-binding protein. Pol gamma has two important functions: synthesizing and proofreading mtDNA. Multiple studies have shown that reactive oxygen species reduce the proofreading capacity of Pol gamma, resulting in increased errors during proofreading. Therefore, oxidation, which exacerbates mtDNA mutations, contributes to erroneous proofreading and subsequently results in mtDNA damage. This suggests that mutations in mtDNA are largely random in nature. Oxidation of Pol gamma may explain the occurrence of mtDNA mutations in senescence. Thus, mtDNA mutations may be closely linked to cardiac senescence [49,50].

Mitochondrial DNA copy number and cardiovascular disease

Mt DNA can predict the progression of cardiovascular disease. Ashar and colleagues reported that mtDNA copy number is negatively correlated with cardiovascular events, with a significantly greater impact on cardiovascular risk assessment in younger individuals [51]. However, these trials had relatively small sample sizes. Therefore, to determine the clinical potential of mtDNA, several factors must be taken into account, including gender, lipid profile, tobacco use, age, blood pressure, and environmental influences [52,53]. Castellani and colleagues assessed 2,507 European Americans and African Americans for atherosclerosis risk and examined the relationship between mtDNA copy number and nuclear DNA methylation [54]. They reported that controlling mtDNA copy number leads to alterations in methylation and gene expression related to cell signaling, thereby influencing the expression of essential genes. Furthermore, mtDNA copy number plays a crucial role in the pathogenesis of cardiovascular disease. It may reflect the extent of mtDNA damage and serve as a marker of mitochondrial function, helping to predict cardiovascular risk. Studies have shown that mtDNA copy number is significantly reduced in the cardiac muscle cells of individuals with heart failure. OS can exacerbate heart failure and ventricular remodeling. ETC in mitochondria is a significant source of free radicals. In a murine model of myocardial infarction, the expression of the PRDX3 gene and mitochondrial transcription factor A in subjects with heart failure can increase mtDNA copy number, slow ventricular remodeling, and improve survival rates [55]. Ikeda and colleagues conducted a study in mice and reported that excessive expression of mitochondrial transcription factor A and Twinkle mtDNA helicase resulted in a doubling of mtDNA copy number and alleviation of injury to cardiac muscle cells [56]. Thus, increasing mtDNA copy number appears to be a novel strategy for treating oxidative stress-induced cardiac damage.

MtDNA copy number varies according to several factors, including oxidative stress, which is a major contributor to the development of cardiovascular disorders. Further studies on the aforementioned factors are needed to gain a comprehensive understanding of the underlying processes. Additionally, understanding how mtDNA copy number helps predict cardiovascular disease progression and exploring its potential therapeutic applications are important areas for further investigation [57].

Mt energy genesis

Mutations and deletions in mitochondrial DNA impair RC function and reduce proton flow, thereby decreasing mitochondrial membrane potential and suppressing adenosine triphosphate (ATP) production. A number of studies have demonstrated that the mutation 3253 T > C in mitochondrial transfer $RNA^{Leu(UUR)}$ reduces the function of mitochondrial

complexes I and V, leading to a 66% reduction in ATP synthesis, decreased mitochondrial membrane potential, and increased reactive oxygen species production [58,59]. Similarly, the mutations 10454 T > C and 10410 T > C in mitochondrial DNA significantly reduced ATP levels and mitochondrial membrane potential, increased reactive oxygen species production, and elevated concentrations of 8-oxo-Guanine and malondialdehyde, while decreasing concentrations of superoxide dismutase (SOD) and glutathione peroxidase (GPx). The mutation 4467 C > A in mitochondrial transfer RNA^{Met} reduced oxygen utilization, decreased mitochondrial membrane potential by approximately 26%, lowered ATP levels by about 26%, and increased reactive oxygen species production by approximately 114% [60,61]. The mutation 15909 A > G in mitochondrial transfer RNA^Thr decreased ATP synthesis and the levels of mitochondrial translation products, while enhancing reactive oxygen species production. Thus, mutations and deletions in mitochondrial DNA contribute to oxidative stress and mitochondrial dysfunction, which may play a role in the progression of cardiovascular disease, hypertension, and atherosclerosis [62,63].

Mt calcium regulation

Mitochondria can influence cytoplasmic calcium concentrations due to mutations in nuclear genes and mitochondrial DNA. This pathway affects calcium absorption by mitochondria through the mitochondrial Ca2+ uniporter. Cells with the mutation 4263 A > G exhibited decreased expression of the mitochondrial Ca2+ uniporter, leading to impaired regulation of calcium absorption and excessive calcium deposition in the cytosol [64,65]. Numerous mutations in GARS, encoded by the nuclear genome, altered the mitochondrial electron transport chain (ETC), assembly genes, tricarboxylic acid cycle enzymes, and proteins involved in fatty acid oxidation, thereby inducing damage to mitochondrial heart muscle cells. In particular, mitochondrial Ca2+ metabolic processes and the interaction sites between the endoplasmic reticulum and mitochondria were altered. This promoted the clinical manifestations of hereditary neuropathy. Furthermore, calcium transfer depends on the availability of adenosine triphosphate, although not directly. Mutations in mitochondrial DNA inhibit ATP production and reduce mitochondrial membrane potential, which may impair calcium regulation, induce apoptosis, and disrupt SMCs function [66,67].

The mitochondrial Ca2+ uniporter is essential for calcium transfer between mitochondria and the sarcoplasmic reticulum (SR) in skeletal and cardiac myocytes. Knockout of BNIP-3, which regulates mitophagy in cardiac muscle cells, results in impaired calcium transport in the endoplasmic reticulum, mitochondrial damage, increased apoptotic processes, and left ventricular fibrosis. FOXO3 promotes BNIP-3 expression in cardiac muscle cells, thereby increasing mitochondrial calcium levels, which results in reduced mitochondrial membrane potential, mitochondrial fragmentation, and increased apoptosis [68,69]. Thus, FOXO3-BNIP-3-mediated calcium regulation contributes to mitochondrial dysfunction in heart failure and may serve as a useful therapeutic target. SERCA-2a contributed to increased calcium flux caused by depolarization, along with elevated calcium deposition in the endoplasmic reticulum and mitochondria of stellate neurons in spontaneously hypertensive rats. Suppression of the mitochondrial Ca2+ uniporter has been shown to inhibit neuronal mitophagy in ischemia-reperfusion injury, thereby revealing a new calcium-mediated defense mechanism [70,71].

Overall, multiple molecular processes are associated with mitochondrial calcium homeostasis and the development of cardiovascular disease. Changes in mitochondrial calcium concentrations affect mitophagy, mitochondrial function, and the processes of fission and fusion, and may represent a promising target for cardiovascular disease therapy [72,73].

Changes in mitochondrial DNA methylation in metabolic and CV disorders

Metabolic syndrome (MS) is a group of conditions that includes hypertension, insulin resistance (IR), abnormal fat distribution, and obesity, all of which are associated with a higher risk of diabetes mellitus and cardiovascular disorders. In addition to other pathological abnormalities that contribute to MS progression, disruptions in mitochondrial genesis and oxidative phosphorylation (OXPHOS) are considered essential; however, the molecular processes at the core of mitochondrial dysfunction have yet to be fully explored. Recent research indicates that mitochondrial dysfunction associated with obesity, IR, diabetes mellitus, and cardiovascular diseases may be due to changes in mitochondrial DNA methylation [74,75].

IR is associated with a decrease in mitochondrial DNA copy number due to increased methylation of the leukocyte displacement loop in patients with obesity. Furthermore, the degree of displacement loop methylation is associated with an increased risk of diabetes mellitus and has been identified as a potential marker for the initial stage of pre-diabetes. Treatment of human retinal endothelial cells (HRECs) with elevated glucose levels increased displacement loop methylation [76,77]. Additionally, the retinal vasculature of patients with diabetic eye disease exhibited similar increases in displacement loop methylation and reductions in mitochondrial DNA transcription. Increased displacement loop methylation was observed in buccal swab DNA samples obtained from women with obesity. Moreover, a specific CpG site in the displacement loop region was associated with body composition impairment, as assessed by BMI, waist-to-height ratio, and bioelectrical impedance analysis. Notably, adverse body composition can be predicted by mitochondrial DNA copy number and displacement loop methylation. Displacement loop methylation in the retinal microvascular tissues of type 2 diabetic (T2D) rats was increased compared to that of type 1 diabetic (T1D) rats or those on a high-fed diet [78,79].

The potential role of impaired mitochondrial DNA methylation in the development of cardiovascular disease was first suggested by research conducted on platelet mitochondria from ten individuals with cardiovascular disease and seventeen controls. The results indicated that elevated methylation levels of the MTTL1, MTCO1, MTCO2, and MTCO3 genes were detected in individuals with cardiovascular disease. Subsequent research conducted on human samples, in cellular cultures, and in mouse models of occlusive disease and aortic stenosis revealed that in vascular smooth muscle cells (VSMCs), the DNMT-1 enzyme translocates to mitochondria in response to pro-proliferative signals, where it enhances displacement loop methylation [80,81]. Increased displacement loop methylation inhibited mitochondrial DNA transcription, contributing to mitochondrial dysfunction and decreased adenosine triphosphate synthesis, which disrupts the ability of VSMCs to contract in the context of occlusion and re-stenosis. Recent research aimed to define the differences in mitochondrial function between stable coronary artery disease and acute coronary syndrome. Mitochondrial DNA copy number and the degree of methylation of PPARGC1A and the displacement loop were assessed in circulating white blood cells from fifty subjects with stable coronary artery disease and fifty subjects with acute coronary syndrome. The results showed that subjects with stable coronary artery disease had elevated mitochondrial DNA copy numbers and reduced displacement loop methylation, indicating that changes in mitochondrial DNA copy number and methylation can influence the phenotype of coronary artery disease [82].

To understand why only some patients with obesity develop cardiovascular disease, the methylation of MTTF, MTTL1, MTCO1, MTCO2, and MTCO3, as well as the displacement loop and MT-OLR, were assessed in platelet mitochondrial DNA from two hundred patients with obesity, of whom eighty-four had CVD. The researchers found that methylation levels of MTTL1, MTCO1, and MTCO3 were elevated in patients with cardiovascular disease, suggesting that methylation of these genes may predict the occurrence of cardiovascular disease in individuals with obesity [83]. Table 2 provides a summary of various DNA alterations that impact cardiac function.

Table 2. Impact of Mitochondrial DNA Alterations on Cardiac Function and Potential Therapeutic Targets

Mutation/Condition	Type of Mutation	Impact on Cardiac Function	Mechanism of Injury	Potential Therapeutic Targets
Point Mutations	Mt 3253 T > C	Decreased ATP production	Impairment of mitochondrial respiratory chain	Enhancing mitochondrial biogenesis
	Mt 4467 C > A	Reduced membrane potential	Increased ROS production	Antioxidant therapies
Deletions	Various deletions	Impaired energy metabolism	Loss of mitochondrial DNA integrity	Gene therapy to restore function
8-oxo-Guanine Accumulation	Result of oxidative stress	Correlates with cardiac dysfunction	Mutation in nucleotide pool	OGG1 and MUTYH pathway modulation
Reduced Mitochondrial DNA Copy Number	Chronic oxidative stress	Increased risk of cardiovascular events	Correlates with heart failure	Strategies to increase mitochondrial biogenesis
Methylation Changes	displacement loop methylation	Impairs mitochondrial function	Inhibits transcription of essential genes	Demethylating agents targeting DNMT-1
Calcium Regulation Abnormalities	Mutation in Mt Ca2+ uniporter	Excess calcium in cytosol	Disruption of calcium homeostasis	Calcium channel modulators

Translation challenges

Research indicates that while mice are valuable for studying cardiac aging and cardiovascular disease treatments, the results often do not translate effectively to humans due to genetic and biological differences. Moreover, interventions such as nutrition and exercise may not yield universal benefits, particularly in individuals who have already optimized these factors, and outcomes can vary based on gender [84,85]. To address these challenges, precision medicine and more clinical trials are essential for customizing strategies and providing reliable evidence for effective aging management in human populations.

Mitochondrial dysfunction plays a pivotal role in cardiac aging, with extensive research illuminating the intricate connections between mitochondrial health, aging, and cardiac disease. As we age, our mitochondria experience significant transformations, including decreased energy production, heightened oxidative stress, and impaired mitophagy [86,87].

These alterations can lead to the accumulation of damaged mitochondria, ultimately contributing to the onset of age-related cardiac diseases [88,89].

Studies indicate that mitochondrial dysfunction is a hallmark of aging in the heart. For instance, significant declines in mitochondrial function and biogenesis have been noted in older individuals [90,91]. Radak et al. highlighted that levels of SIRT3, a crucial regulator of mitochondrial function, diminished in the skeletal muscles of the elderly. [92] This decline correlates with impaired mitochondrial metabolism and mitophagy, underscoring SIRT3's essential role in maintaining mitochondrial integrity [93].

Moreover, other key regulators of mitochondrial function, such as PGC-1α and NRF1, also exhibit age-related declines. These losses can trigger a cascade of negative effects, including impaired mitochondrial biogenesis, reduced energy output, and increased oxidative stress, which may facilitate the progression of age-related cardiac issues like heart failure and arrhythmias [94,95].

Further research has established that mitochondrial DNA (mtDNA) copy number serves as a crucial predictor of cardiovascular disease (CVD) progression [96,97]. Findings by Ashar et al. demonstrated a negative correlation between mtDNA copy number and cardiovascular events, with pronounced implications for younger individuals, despite the trial's relatively small sample size [51]. Thus, it's imperative to consider various factors—such as gender, lipid profiles, tobacco use, age, blood pressure, and environmental conditions—when evaluating mtDNA's clinical potential.

In a study by Castellani and colleagues involving 2,507 European and African Americans, the relationship between mtDNA copy number and nuclear DNA methylation was examined [54]. They discovered that controlling mtDNA copy number affects methylation patterns and gene expression related to cell signaling, thereby influencing the expression of essential genes [54]. Notably, reduced mtDNA copy number has been observed in cardiac muscle cells of heart failure patients, emphasizing its importance in cardiovascular disease pathogenesis [98].

OS can exacerbate heart failure and trigger ventricular remodeling. The mitochondrial ETC is a significant source of free radicals. A murine model of myocardial infarction revealed that the expression of the PRDX3 gene and mitochondrial transcription factor A can increase mtDNA copy number, slow down ventricular remodeling, and improve survival rates [99,100]. Ikeda et al. reported that excessive expression of both mitochondrial transcription factor A and Twinkle mitochondrial DNA helicase can double mtDNA copy number and alleviate cardiac muscle cell injury [56]. Therefore, elevating mtDNA copy number represents a promising strategy for treating OS-induced cardiac damage.

It is crucial to understand that mtDNA copy number varies based on numerous factors, including oxidative stress, a major contributor to cardiovascular disorders. Further research into these factors is essential for unraveling the underlying mechanisms. Additionally, exploring how mtDNA copy number can predict CVD progression and its potential therapeutic applications are vital areas for future investigation [101,102].

Fortunately, emerging research has pinpointed several therapeutic targets aimed at mitigating mitochondrial dysfunction and promoting healthy aging. For instance, nicotinamide riboside (NR), a substrate in the NAD+ salvage pathway, has shown promise in enhancing mitochondrial function and reducing oxidative stress in older adults [103,104]. Similarly, metformin, a widely used antidiabetic medication, has demonstrated the ability to promote mitochondrial biogenesis and boost energy production in aging populations [105,106].

In conclusion, mitochondrial dysfunction is a significant contributor to cardiac aging. Ongoing research continues to reveal potential therapeutic targets for addressing this dysfunction. By deepening our understanding of the complex relationships between mitochondrial function, aging, and cardiac disease, we can devise more effective strategies for promoting healthy aging and preventing age-related cardiac diseases.

Conclusion

The intricate relationship between mitochondrial (Mt) dysfunction and cardiovascular diseases (CVD) has been extensively explored, highlighting the pivotal role of Mt dysfunction in the pathogenesis of CVD. The evidence presented underscores the significance of understanding the molecular processes underlying cardiac aging, including Mt DNA mutations, Mt genome organization, Mt dynamics, Mt DNA copy number, Mt energy generation, Mt calcium regulation, and Mt DNA methylation.

One of the key findings is the importance of Mt DNA copy number as a predictive marker for CVD progression. Our analysis of the literature suggests that Mt DNA copy number is a critical factor in the development of CVD, and that alterations in Mt DNA copy number may be a useful diagnostic marker for CVD. Furthermore, the potential of Mt DNA methylation as a therapeutic target in the context of CVD is highlighted.

The insights gained from this review have significant implications for future research in the field of cardiac aging and Mt dysfunction. Future studies should aim to unravel the complexities of Mt dysfunction in the context of cardiovascular aging, offering opportunities to develop targeted interventions that could potentially mitigate the impact of aging on cardiovascular health. Additionally, further research is needed to explore the potential of Mt DNA copy number and Mt DNA methylation as diagnostic markers and therapeutic targets in the context of CVD.

The relationship between Mt dysfunction and CVD is complex, and understanding the molecular processes underlying cardiac aging is crucial for developing effective treatments. Enhancing mitochondrial biogenesis, increasing mitochondrial DNA copy number, modulating mitochondrial DNA methylation,

and targeting calcium regulation are potential therapeutic strategies that may be useful in the context of CVD. However, further research is needed to fully understand the implications of these processes and to develop targeted interventions.

Understanding the influence of Mt dysfunction on CVD pathogenesis offers a promising avenue for the development of precision medicine approaches tailored to mitigate the effects of cardiac aging and its associated cardiovascular risks. As research into the molecular processes of cardiac aging and Mt dysfunction continues to advance, it is essential to further explore the implications of these processes for diagnostic, prognostic, and therapeutic purposes.

In summary, the intricate interplay between cardiac aging and Mt dysfunction presents a rich landscape for continued research and exploration, with the potential to yield innovative strategies for the prevention, diagnosis, and treatment of cardiovascular diseases. The insights provided in this review lay the foundation for further inquiry and development in the field of cardiac aging and Mt dysfunction, offering promising prospects for improving cardiovascular health outcomes in aging populations.

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