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AHA SCIENTIFIC STATEMENT

Mitochondrial Genetics in Cardiovascular Health and Disease: A Scientific Statement From the American Heart Association

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ABSTRACT: Metabolic and genetic abnormalities have long been noted in cardiovascular diseases, but the contribution of mitochondrial genetic (mitochondrial DNA [mtDNA]) variation is understudied. Mitochondrial genetics is complex in that each mitochondrion contains multiple mtDNA copies that may carry different variants, which is called heteroplasmy. Heteroplasmic variation is dynamic, increases with advancing age, and may contribute to aging-related cardiovascular diseases. Pathogenic variants in mitochondrial genes of the mtDNA or nuclear genome cause mitochondrial diseases, often with cardiac involvement, particularly in patients with adult-onset disease. Population-level studies have identified mtDNA variants associated with cardiovascular risk factors and disease, but evaluation of mtDNA genetic variation is often limited to only a handful of variants and small sample sizes. Studies in animal models have linked several mtDNA variants to cardiac remodeling and dysfunction and suggest a role for mitochondrial–nuclear genetic interactions in disease penetrance. The objective of this scientific statement is to outline the current state of understanding of the role of mitochondrial genetics in cardiovascular pathobiology and highlight important gaps in knowledge. The intended audience of this scientific statement is meant to be broad, spanning clinical, translational, and basic researchers and health care professionals. Despite remaining limitations and barriers, recent advances in genomic sequencing, mtDNA gene editing modalities, and the directed differentiation of stem cells to cardiovascular cell types are creating new opportunities to advance understanding of mitochondrial genetics in cardiovascular pathophysiology.

Key Words: AHA Scientific Statements ■ cardiovascular diseases ■ DNA, mitochondrial ■ genes, mitochondrial ■ mitochondria

The human heart consumes 6 grams of ATP a day, most of which is generated by oxidative phosphorylation (OXPHOS).[1,2] The core components of OXPHOS complexes I, III, IV, and V are encoded by the mitochondrial genome (mitochondrial DNA [mtDNA]), along with the functional RNAs necessary for their expression (22 tRNAs and 2 ribosomal RNAs). The nuclear genome encodes OXPHOS complex II in its entirety, as well as additional subunits and assembly factors for the other OXPHOS complexes. The nuclear genome also encodes proteins involved in mitochondrial replication and transcription, metabolic processes housed within mitochondria, transport across mitochondrial membranes, mitophagy (ie, the targeted clearance of mitochondria through the ubiquitin–proteasome system), apoptosis, and heme biosynthesis. Coordination between the mitochondrial and nuclear genomes is required to form functional OXPHOS complexes, enabling optimal ATP synthesis.[3]

Defects in the mitochondrial genes affecting either the nuclear genome or mtDNA cause mitochondrial diseases that are typically multisystemic in presentation and often affect the heart. mtDNA variants may be homoplasmic, with all mtDNA copies carrying the same allele; or heteroplasmic, whereby different mtDNA copies carry different variants (Figure 1). Heteroplasmic variants may be maternally inherited or somatic. Pathogenic mtDNA variants that cause rare mitochondrial

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/CIR.0000000000001393

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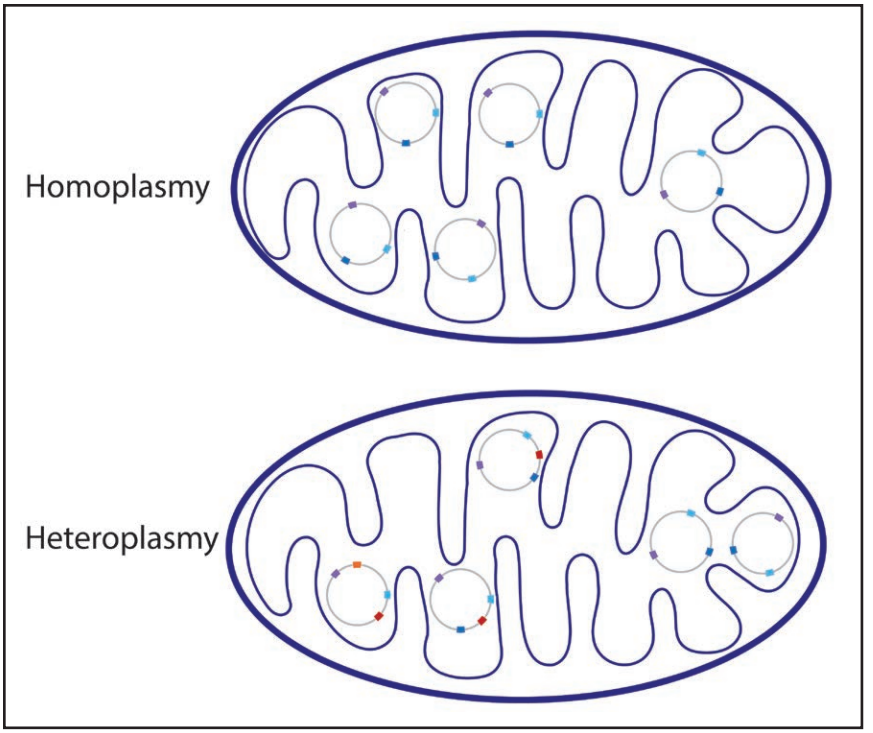

Figure 1. Homoplasmic and heteroplasmic mtDNA variants.
Due to the multiple copies of mitochondrial DNA (mtDNA; gray circles) in a mitochondrion, mtDNA variants may be either homoplasmic or heteroplasmic. Homoplasmic variants (illustrated by the blue and purple marks on the mtDNAs) are present at variant allele frequencies (VAFs) of 100% and typically are common population-level polymorphisms, such as those that define haplogroups, which may interact with other genetic and environmental risk factors to confer disease risk. Heteroplasmic variants (illustrated by the orange and red marks on the mtDNAs) are present on some of the mtDNA copies but not all copies in a mitochondrion or cell. In the example in the bottom panel, the red heteroplasmic variant is present at a VAF of 60% and the orange heteroplasmic variant at a VAF of 20%. Heteroplasmic variants may be acquired or maternally inherited. Heteroplasmic variants may be rare mtDNA disease-causing variants (often with cardiovascular involvement) or present at low levels with unknown significance. The role of heteroplasmic variants in more prevalent cardiovascular diseases is unclear.

syndromes often exist in a state of heteroplasmy, with levels of the variant being dynamic and variable across cells or tissues (Figure 2). Heteroplasmic mtDNA variation is widespread in the general population, and higher levels of heteroplasmic variants are associated with advancing age.[4,5] The accumulation of heteroplasmic mtDNA variants over the human life span is thought to contribute to aging-related diseases, including more prevalent cardiovascular diseases (CVDs).[5–9] Several mitochondrial haplogroups, consisting of collections of similar mtDNA sequences that contain haplogroup-defining homoplasmic variants, are associated with CVD and CVD risk factors, and modify the penetrance of some pathogenic mtDNA variants.[10–12] Few population studies have evaluated the association of mtDNA variants beyond select variants with measures of cardiovascular function and structure, cardiovascular risk factors, or disease.

Methodologic limitations have created barriers to our understanding of the mechanisms of mtDNA variation in cardiovascular biology. Advances in next-generation sequencing (NGS), gene editing tools, and stem cell biology now provide new opportunities to advance our understanding of the contribution of mtDNA variation in cardiovascular health and disease. Now that NGS is readily available for both diagnostics and research, best practices for identifying and defining mtDNA variants—particularly heteroplasmic mtDNA variants—are needed, and are outlined herein. Advances in gene editing technologies are starting to facilitate and accelerate mechanistic studies of mtDNA variation in both cellular and animal models. We summarize our current understanding of the role of mitochondrial genetic variation in CVD and identify key gaps in the field.

BEST PRACTICES FOR IDENTIFYING mtDNA VARIANTS

In Table 1, we outline considerations and suggested quality control procedures for different sequencing platforms for studying mitochondrial genetics. Whole genome sequencing (WGS) allows for the identification of mtDNA variants at unprecedented levels, facilitating population-level and mechanistic studies of mtDNA variation and improved diagnosis of mitochondrial disease.[13] Because of the multicopy nature of mtDNA, sequencing depth for the mitochondrial genome is typically much greater than for the nuclear genome, which permits variant identification at low variant allele frequencies (VAFs; typically 3%–10%, depending on the cell or tissue type, sequencing method, and depth). VAF thresholds are typically used to define heteroplasmic versus homoplasmic variants, considering the potential for artifacts at low VAFs. No consensus exists on the appropriate VAF threshold for defining heteroplasmic variants, which should be carefully considered to account for sequencing errors and depth.

A typical rigorous bioinformatic pipeline used for detecting mtDNA variants in WGS data sets is represented in Figure 3, along with recommendations for quality control procedures and considerations. Sequencing reads are first mapped to a reference genome and the circularity of the mtDNA should be considered to increase mapping around the arbitrary cut sites that linearize the

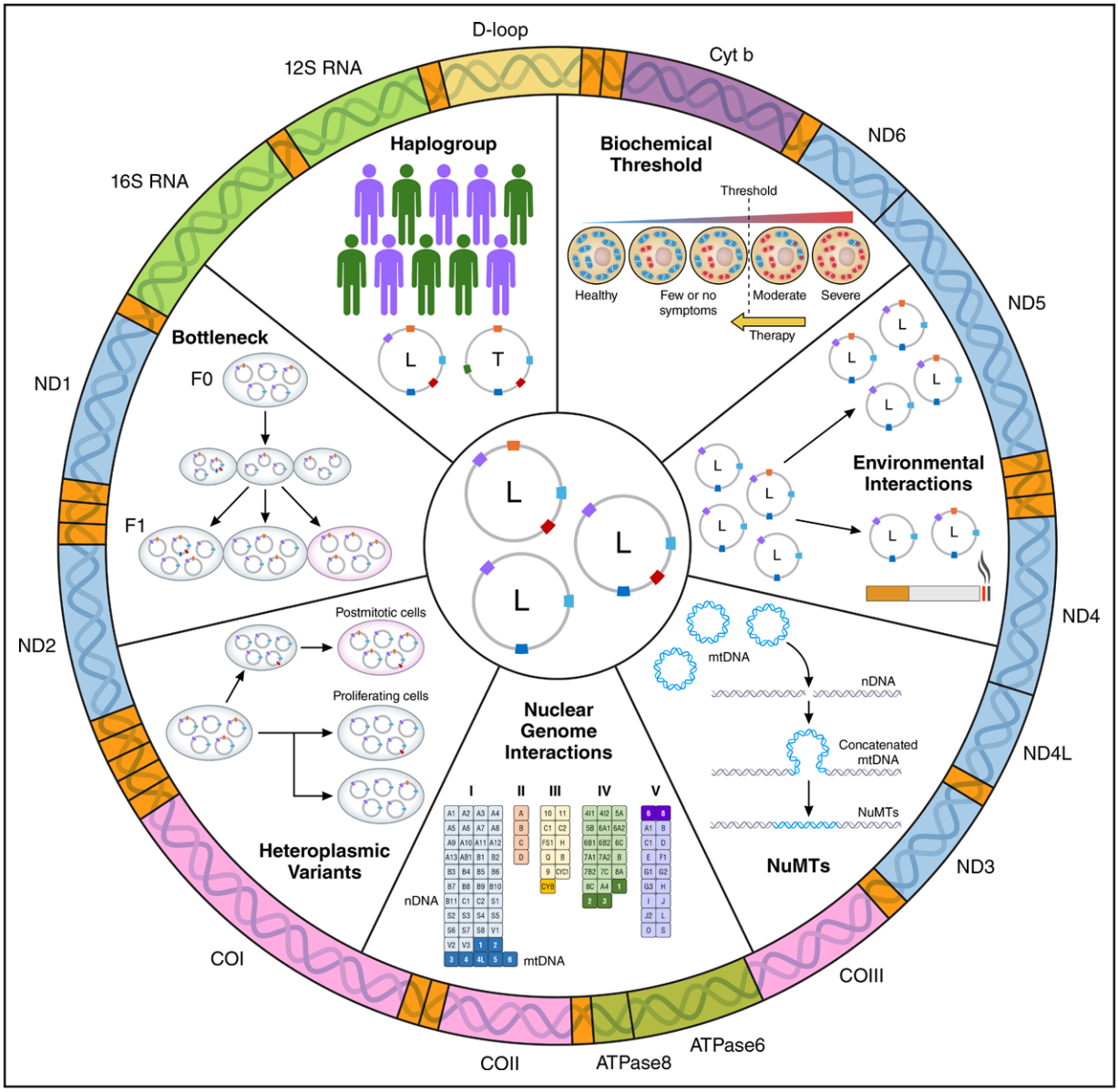

Figure 2. Unique mtDNA characteristics and the complexities of mitochondrial genetics.
The outer track represents the structure of the double-stranded, circular mitochondrial DNA (mtDNA), with the noncoding region (D-loop) in yellow, tRNA genes in orange, ribosomal RNA genes in bright green, and oxidative phosphorylation (OXPHOS) complex I genes in blue, complex III gene in purple, complex IV genes in pink, and complex V genes in green. The innermost circle contains representative mtDNAs from an individual belonging to haplogroup L, defined by the purple homoplasmic "variant." The mtDNAs in the innermost circle also carry 2 variants in a heteroplasmic state in orange and red. The mtDNAs carrying different variants in the innermost circle are used to illustrate unique aspects of the mtDNA in the wedges of the middle track. Starting at the top left, every individual in a population belongs to a mitochondrial haplogroup, defined by several specific mtDNA variants reflective of prehistoric human migration patterns, with 2 haplogroups (L defined by the purple and T by the green variant) depicted. Moving clockwise in the middle track, pathogenic heteroplasmic variants must reach a particular variant allele frequency (VAF) before a change in cellular phenotype occurs, termed the biochemical threshold, which determines mitochondrial disease onset. A key targeting strategy of gene editing strategies or therapies is to increase the number of copies of mtDNAs carrying the referent allele or decrease the mtDNAs with the pathogenic variant to levels below the biochemical threshold. Environmental factors, including cigarette smoking and alcohol consumption, result in changes in the number of mtDNA copies carrying a pathogenic variant, thereby affecting disease penetrance. A key challenge in identifying mtDNA variants and accurately determining the VAFs of heteroplasmic variants relates to nuclear mtDNA segments (NuMT), which are pieces of the mtDNA that have been inserted into the nuclear genome. Although poorly understood, the penetrance of mtDNA variants is likely affected by other genetic interactions, as OXPHOS complexes I, III, IV, and V are encoded by both the mtDNA and nuclear genome. Heteroplasmic mtDNA variants may undergo expansion in nonproliferative cells because mtDNA replication is not tied to the cell cycle (ie, relaxed replication). In proliferating cells, heteroplasmic variants are randomly segregated, resulting in daughter cells with different VAFs, and may undergo selection in a cell type–specific manner. Heteroplasmic variants, particularly pathogenic variants, may also be selected against through purifying selection in some proliferative cell types. Heteroplasmic variants can be transmitted through the maternal lineage with random segregation into the daughter cells, resulting in some offspring with high enough levels of a pathogenic mtDNA variant to result in disease, whereas other daughter cells and resulting offspring may have low levels of the pathogenic variant and not develop disease.

Table 1. Next-Generation Sequencing Considerations and Recommendations

Factors	Considerations and recommendations
Preanalytical	
Tissue or cell type	Determine DNA input available (most platforms require 30–50 ng)
	Consider relevance to the question being asked: disease-causing variants may be lost in blood cells, particularly in adults
Sample storage	Minimize freeze–thaws and maintain samples on ice during processing
	Buffer/inhibit nucleases to minimize degradation
	Note platform-specific requirements (eg, regarding pH, double-stranded vs single-stranded DNA)
Sequencing method	
WGS	Captures both nuclear and mitochondrial genomes
	Depth of 30× is sufficient for variant detection in nuclear genome, but mtDNA requires greater depths (500–1000×) to detect heteroplasmic variants, particularly at low VAFs
	Ideal method for identifying SNVs across both genomes and coding and noncoding regions alike
	Often unable to identify structural variants with high accuracy
	mtDNA copy number can be estimated
WES	Captures SNVs in both the nuclear and mitochondrial genomes but is restricted to coding regions and may miss important variants in noncoding regions; some platforms do not include baits for mtDNA
	Data easier to interpret because annotation for noncoding regions is more limited (particularly for the nuclear genome)
	Often does not obtain high depth of mtDNA to detect heteroplasmic variants, particularly those at low VAF
	Typically unable to identify structural variants with high accuracy
	Cannot estimate mtDNA copy number
Long-read WGS	Sequencing of individual mtDNAs in their entirety with a single read, allowing for phasing of mtDNA variants (identification of variants on the same mtDNA molecule)
	Better detection of deletions and complex rearrangements over other NGS methods
	Superior accuracy for homopolymeric regions compared with other sequencing methods
	High inaccuracy for indels
	Optimal depths have yet to be defined
	Minimizes NuMT contamination
	Facilitates sample-specific consensus reference sequence generation, which minimizes reference sequence biases
Enrichment of mtDNA	
Mitochondrial isolation	Isolation of mitochondria before DNA isolation enriches for mtDNA while minimizing NuMT contamination
PCR-based	High depth obtained for mtDNA
	May introduce biases depending on the location of primers
	Introduces sequencing errors (varies across different polymerases) that may be misidentified as low-level heteroplasmic mtDNA variants
	Short amplicon bias that may exaggerate the VAF of heteroplasmic deletions
Capture-based	Some platforms may not include baits for mtDNA
Degradation of linear DNA	Enzymatic degradation of linear DNA enriches for the mtDNA, which is circular
	Decreases the risk of NuMT contamination
	If a variant is present near the cut site, it may result in selection bias, which can be overcome by using different enzymes on subsamples
Analytical	
Reference genome	
rCRS	Sequence of an individual of European ancestry
	Introduces biases in variant identification for samples of less similar ancestries
Yoruban reference	Sequence of an individual of African ancestry
	Introduces biases in variant identification for samples of less similar ancestries
RSRS	Artificial sequence of an individual of African ancestry
	Introduces biases in variant identification for samples of less similar ancestries
Consensus reference	Each individual sample has its own consensus reference built from the variants present at a VAF of ≥50%
	Increases read mappability and ability to detect low-level heteroplasmic variants

(*Continued*)

Table 1. Continued

Factors	Considerations and recommendations
Base quality score	Set a minimum score requirement (usually 30)
Coverage	Determine a minimum threshold for coverage across samples and require a certain percentage of bases with a minimum depth for sample inclusion Set a minimum requirement for positional depth
Read mapping	Define a minimum requirement for % reads aligned and % unique reads for sample inclusion
Strand imbalance	Variants with a strong strand bias may be sequencing errors
NuMT contamination	If preanalytical methods are not applied to limit NuMT contamination, exclude paired reads that map to both the nuclear and mitochondrial genomes and filter based on mapping quality scores Filtering variants from known NuMT sequences is insufficient, particularly if low-level heteroplasmic variants are of interest
VAF threshold	Sensitivity for low-level heteroplasmic mtDNA variant detection should be carefully evaluated considering the sequencing method used, sample and positional depth, and polymerase (if PCR-based) and sequencing error rates
Postanalytical	
Data sharing	Open vs restricted access, depending on the data set Include details on sequencing measures and metadata
Validation	
SNVs	Sanger sequencing or amplicon sequencing
Single large-scale deletions and duplications	Validation with long-range PCR or digital droplet PCR methods
Multiple deletions and rearrangements	Southern blot is the ideal validation approach

mtDNA indicates mitochondrial DNA; NGS, next-generation sequencing; NuMT, nuclear mtDNA segment; PCR, polymerase chain reaction; rCRS, revised Cambridge Reference Sequence; RSRS, Reconstructed Sapiens Reference Sequence; SNV, single nucleotide variant; VAF, variant allele frequency; WES, whole exome sequencing; and WGS, whole genome sequencing.

circular chromosome. The revised Cambridge Reference Sequence historically has been used as the reference genome for mtDNA variant identification[14]; however, its use has been questioned, because the revised Cambridge Reference Sequence was derived from an individual of European ancestry, introducing biases when analyzing mtDNA variants from non-European populations. Other mtDNA reference sequences based on individuals of African ancestry have been proposed, but introduce similar biases when identifying variants from individuals with less similar mitochondrial sequences.[15,16]

Generation of a consensus reference sequence, where a reference sequence is generated for each individual sample, circumvents the biases introduced by the use of a single reference sequence. Use of consensus reference sequences increases read mapping and depth, allowing for greater sensitivity in the detection of low-level heteroplasmic variants,[17] but involves a tradeoff: generating the consensus reference is more computationally intensive than using the same reference sequence for all samples and requires position standardization. Position numbering is standardized to that of the revised Cambridge Reference Sequence to maintain position numbering consistency with previous literature and mtDNA databases.

Nuclear mtDNA segments (NuMTs) are prevalent contaminants in variant calling studies that must be considered when identifying mtDNA variants in NGS data sets.[18–20] NuMTs are fragments of the mtDNA that have been integrated into the nuclear genome (Figure 1).[18] NuMTs range in size up to nearly the entire length of the mtDNA, or longer, because NuMTs may be duplicated.[18,21] Some NuMTs are common within the population, but many are rare or unique to individuals; hence, merely filtering population-level annotated NuMTs is likely inadequate if NuMT sequence variants are to be excluded from analysis of low-level mtDNA heteroplasmy.[20] Setting higher VAF thresholds is also insufficient for filtering NuMT-derived false-positive mtDNA variants[20]; hence, ideally, NuMT-derived reads should be identified and filtered before read alignment to a reference genome.

The mtDNA variant callers have a high concordance for identifying homoplasmic variants, but vary in their false-positive and false-negative detection rates for heteroplasmic variants, particularly for variants at low VAF and those located in hypervariable or homopolymer regions, where alignment errors can occur.[17] Bioinformatic methods for the accurate identification of structural mtDNA variants must be used with caution, because indel calls are often discordant between variant callers. Machine learning algorithms, such as MitoScape or MSeqDR, are advancing toward the accurate identification of structural mtDNA variants.[22,23]

Bioinformatic functional predictions and scoring systems have been developed specifically for mtDNA variants to aid in the interpretation of variants associated with clinical phenotypes (Table 2). A key challenge in the field is that the VAF threshold required for an mtDNA variant

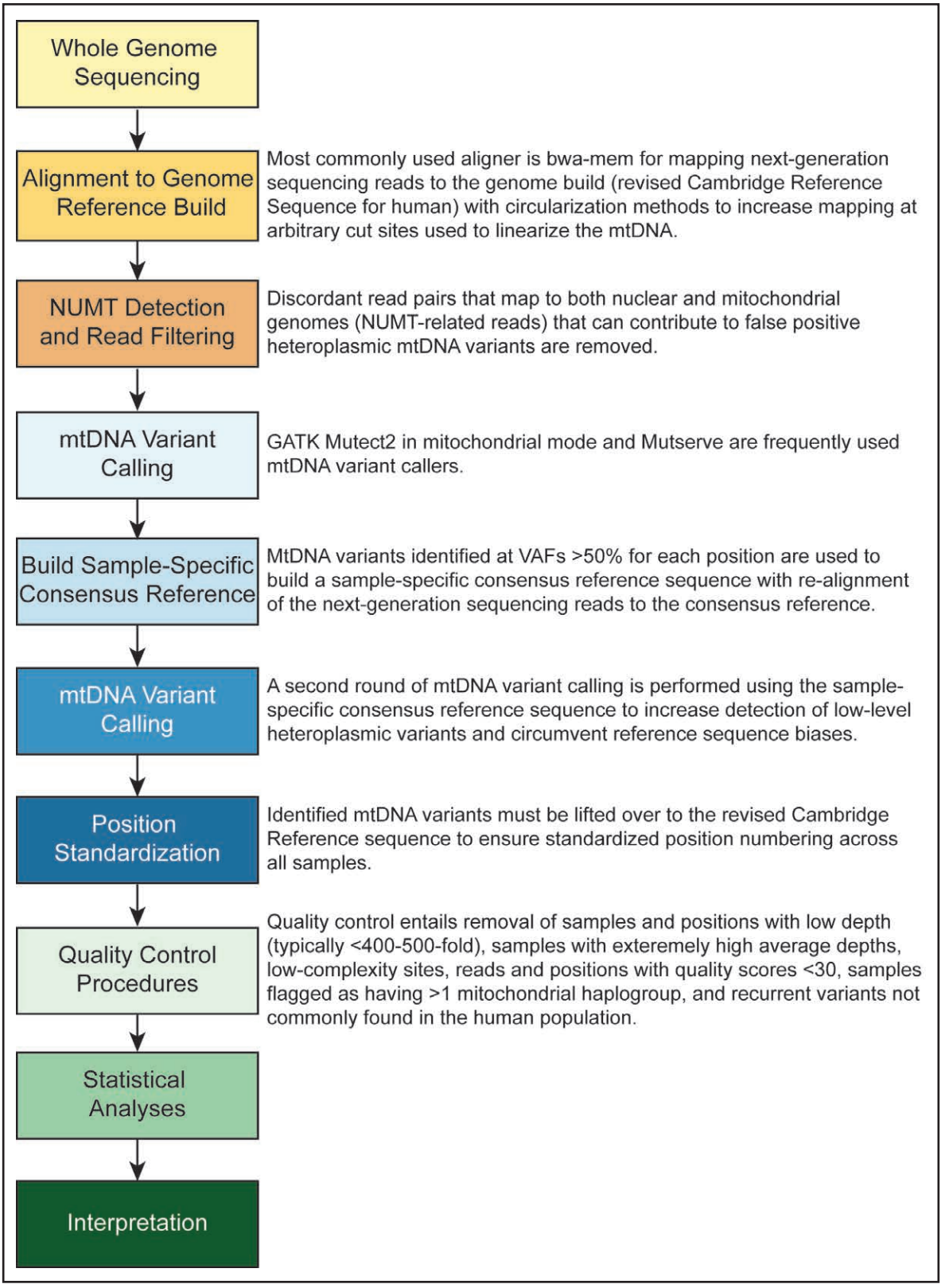

Figure 3. Bioinformatic pipeline for mtDNA variant identification in whole genome sequencing data sets.
Next-generation sequencing reads are aligned to a human genome build (containing the revised Cambridge Reference Sequence) for both detection of nuclear mtDNA segments (NuMTs; see Wei et al[18]) and identification of mitochondrial DNA (mtDNA) variants, typically using bwa-mem as the aligner of reads to the reference genome. Circularization of the reference genome is important for increasing read mapping.[17,234] After filtering NuMT-related reads, the mtDNA variants identified in the first iteration of variant calling are used to construct a consensus reference sequence for each individual sample. A second round of mtDNA variant calling is performed using the consensus (*Continued*)

Figure 3 Continued. reference sequence for each sample and the sequence positions are standardized to that of the revised Cambridge Reference Sequence through a position liftover step. Commonly used mtDNA variant callers are GATK Mutect2 in mitochondrial mode and Mutserve.[17,234] Rigorous quality control procedures must be performed, including filtering out samples and positions with low depth (usually samples and positions with <400- to 500-fold), samples with extremely high average depths, low-complexity sites (positions 66–71, 301, 302, 310, 316, 3107, 12418–12425, and 16182–16194), reads and positions with quality scores <30, samples flagged as having >1 mitochondrial haplogroup (indicates possible sample contamination; commonly used tools include Haplocheck[235] and Haplogrep[236]), and recurrent variants not commonly found in the human population (per population allele frequencies on gnomAD[234] or HmtDB[237]). The average depth of the samples and depth by locus must be considered when defining the heteroplasmic and homoplasmic VAF cutoffs before statistical analyses.

to exert an effect on mitochondrial function (termed the biochemical threshold; see Figure 1), or cause disease (phenotypic threshold), is largely unknown outside of variants in tRNA-encoding genes (typically VAFs >60%) and in cell types relevant to CVDs.[24,25]

MITOCHONDRIAL DISEASES

Mitochondrial diseases are multisystemic syndromes with highly variable clinical presentations, even within a specific diagnosis. Neurologic manifestations are the most common presenting features, but ≈30% of individuals with genetically confirmed mitochondrial disorders have associated cardiovascular issues.[26,27] The heart and brain are the most frequently affected tissues in mitochondrial diseases because of their high energy requirements. The cardiovascular presentations observed in mitochondrial diseases include both cardiac arrhythmias and cardiomyopathy. CVD is the most common cause of death among adult patients with mitochondrial disorders, and appropriate diagnosis of a mitochondrial disease can have important implications for cardiac care.[28]

The diagnosis of mitochondrial disorders is complicated by both phenotype and genotype complexity. From a phenotype standpoint, the broad variability and overlap in symptoms among and across diagnoses, and the occasional nonsyndromic single-tissue presentation, can make targeted testing difficult. In addition, mitochondrial genetics is complex because the underlying variants may occur in mitochondrial genes encoded by either nuclear DNA (Table 3) or mtDNA (Table 4). Furthermore, mtDNA disorders follow non-Mendelian inheritance principles (eg, heteroplasmy, bottleneck effects, phenotypic or biochemical thresholds; see Figure 2),[24,29,30] and variant classification remains challenging because the mtDNA is highly polymorphic in the population.

How a patient presents often dictates the approach to genetic testing. In cardiogenetic clinics, gene panels are the norm. However, most cardiomyopathy and arrhythmia gene panels do not include screening for mitochondrial genes on either the nuclear or mitochondrial genomes. Therefore, if a mitochondrial disorder is suspected on the basis of clinical presentation and family history, mindful selection of the test, and testing laboratory, is crucial to diagnosis. mtDNA analysis including or followed by whole exome sequencing, soon to be replaced by WGS, is recommended as first-line genetic testing for suspected mitochondrial diseases, rather than panels.[31–34]

When pursuing genetic testing, it is important for health care professionals and for patients and their families to be aware of the clinical implications, as well as the potential emotional, ethical, and legal impact, of the results. Genetic testing—especially larger tests, like

Table 2. Bioinformatic Functional Predictions of mtDNA Variants and mtDNA Databases

Database or scoring method	Description
gnomAD[234]	Population allele frequencies for mtDNA variants
HmtDB[237]	Population allele frequencies for mtDNA variants
MitImpact[238,239]	APOGEE 2 pathogenicity predictions for all possible mtDNA missense variants
MITOMAP[240]	Haplogroup-defining variants and mtDNA phylogenetic trees mtDNA reference sequences MitoTIP tRNA pathogenicity scoring Database of reported polymorphic and disease-causing mtDNA variants
MitoVisualize[241]	Visualization and annotation of variants in mtDNA mtDNA-encoded RNA structural information
MSeqDR mvTool[23]	Database of disease-causing mtDNA variants mtDNA variant annotation
Mitochondrial local constraint scores[242]	Calculated predicted effect of base substitutions Can calculate sum score of all heteroplasmic variants for an individual in a data set
OMIM	Database of variants in mtDNA-encoded genes that cause disease

mtDNA indicates mitochondrial DNA; and OMIM, Online Mendelian Inheritance in Man.

Table 3. Disorders Due to Variants in Nuclear DNA–Encoded Mitochondrial Genes With Cardiac Manifestations

Disease or gene	Age at onset	Prognosis	Cardiac manifestations	Genetic inheritance	Genes/variants	References
Barth syndrome	<1 y	70% survival with mean life expectancy of 40 y	DCM, HT, EFE, LQT, WPW, SVT, VT, SCD	X-linked	*TAFAZZIN*	58,243–247
Complex I deficiency	Childhood		HCM, DCM in isolation or part of multisystem disease (ie, Leigh syndrome)	AR	>30 genes including nuclear DNA-encoded subunits and assembly factors	248,249
Complex II deficiency	Infancy	Substantial mortality in infancy	HCM, DCM, HT, EFE	Primarily AR; 1 AD case in *SDHA* associated with p.Arg451Cys	*SDHA*, *SDHB*, *SDHD*	248,250,251
Complex IV deficiency			DCM, HCM, histiocytoid CMP	AR	*COX6B1*, *SURF1*, *SCO2*	248
Complex V deficiency	Neonatal to childhood		HCM, DCM, HF, WPW, PPHN with multisystemic disease	AR	*TMEM70*; other ATP synthase genes	248,252
DCMA syndrome	<3 y	Most deaths by 15 mo	DCM, HT, LQT	AR	*DNAJC19*; homozygous truncations including Hutterite founder sequence variation IVS3-1 G>C	56,253–255
Friedreich ataxia	Childhood	Mean life expectancy 40 y	HCM, DCM, HF, SVT, AF	AR	*FXN*; triplet repeat expansion	256–259
GRACILE syndrome	In utero	50% die within first 4 mo	LQT, reduced levels of complex III in myocardium postmortem	AR	*BCSIL*; homozygous point sequence variation A232G	260,261
PPA2–sudden cardiac failure	Infancy or early adulthood; extreme alcohol hypersensitivity; virally triggered		SCD, VT, DCM, inflammatory myocarditis, conduction defects	AR	*PPA2*	55,58,262
Leigh syndrome	<1–2 y	Rapid progression to death after onset	HCM (most common), DCM, pericardial effusion, LQT, WPW	Primarily AR, except X-linked in *PDHA1*, *PDHB*, and *PDHX*	>100 genes; of particular risk for cardiac features are complex I and IV genes including *SURF1*, *C12orf65*, *COX10/11/15*, *FOXRED1*, *GFM1*, *LRPPRC*	248,249,263,264
mtDNA depletion syndromes	*ANT1/SLC25A4* in infancy to adulthood; *AGK* (Sengers), *FBXL4*, *OPA1*, *TK2* in infancy; *MGME1* in childhood; *TWNK* (C10orf2) in adulthood; *TYPM* (MNGIE) in adolescence to adulthood		*ANT1*, *OPA1*, and *TK2*: HCM; *AGK*: HOCM, SCD; *FBXL4*: HCM; *MGME1*: DCM; *TWNK*: SCD, PVCs, DCM; *TYPM*: CMP, LQT, SVT, SCD	AD in *ANT1* and *TWNK*; AR for *ANT1*, *AGK*, *FBLX4*, *MGME1*, *OPA1*, *TK2*, *TYPM*	*ANT1/SLC25A4*, *AGK*, *FBXL4*, *OPA1*, *TK2*, *MGME1*, *TWNK*, *TYPM*, *OPA1*	248,260,265–269
NDUFB11 deficiency	Infancy	Substantial mortality in infancy	VF, VT, histiocytoid CMP, DCM, SCD	X-linked	*NDUFB11*	270,271
Nuclear-encoded tRNA genes	*AARS2*: fetal to infancy; *GARS1* in childhood; *KARS1* and *YARS2* in infancy	Substantial mortality with *AARS2*	All HCM; *AARS2* also histiocytoid CMP; *GARS1* also WPW	AR	*AARS2*, *GARS1*, *KARS1*, *YARS2*	272–276

AD indicates autosomal dominant; AF, atrial fibrillation; AR, autosomal recessive; CMP, cardiomyopathy unspecified; DCM, dilated cardiomyopathy; DCMA, dilated cardiomyopathy with ataxia syndrome; EFE, endocardial fibroelastosis; GRACILE, growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death; HCM, hypertrophic cardiomyopathy; HF, heart failure; HOCM, hypertrophic obstructive cardiomyopathy; HT, hypertrabeculation; LQT, long QT syndrome; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; PPHN, persistent pulmonary hypertension of the newborn; PVC, premature ventricular contraction; SCD, sudden cardiac death; SVT, supraventricular tachycardia; VT, ventricular tachycardia; and WPW, Wolff-Parkinson-White syndrome.

whole exome sequencing and WGS—can identify DNA variants of uncertain clinical significance and secondary findings and lead to numerous challenging ethical situations.[35,36] Guidelines from the ACMG around the reporting of secondary findings are available and legislation exists to minimize the risk of genetic discrimination and protect the privacy of genetic information; however, there are limitations that should be discussed with patients.[37,38] A comprehensive pretest consent discussion with the patient and family, ideally with the involvement of a

Table 4. mtDNA Syndromes With Cardiac Manifestations

Disease (prevalence)	Age at onset	Prognosis	Cardiac manifestations	Genes/variants	References
Complex I deficiency	Typically infancy, but varies from prenatal to adulthood		HCM, isolated or with multisystem disease (Leigh syndrome)	*MT-ND1*, *MT-ND2*, *MT-ND4*, *MT-ND5*, *MT-ND6*	248
Complex III deficiency	Infancy		Histiocytoid CMP, DCM, HCM; isolated or multisystemic CMP	m.15498G>A and m.14849T>C in *MT-CYB*	248,277,278
Complex IV deficiency			DCM, HCM, histiocytoid CMP	*MT-CO1*, *MT-CO2*, *MT-CO3*	248
Kearns-Sayre syndrome	<20 y		Cardiac manifestations in up to 50% and SCD reported in up to 20% due to heart block; DCM, LBBB/RBBB, heart block, torsades de pointes, SCD	Large mtDNA deletion that is typically sporadic	279–282
Leigh syndrome	Typically infancy, but varies from prenatal to adulthood		HCM, DCM, conduction block in the context of multisystem disease	16 mtDNA genes with *MT-ATP6*, mtDNA complex I genes, and mt-tRNAs of note for higher cardiac manifestation risk	263,264
LHON (1:30 000–50 000; more common in males)	2–87 y	In adulthood mortality risk is doubled compared with the general population	HCM, HT, WPW, SND, VT, SCD	90% of cases caused by m.11778G>A in *MT-ND4*, m.3460G>A in *MT-ND1*, and m.14484T>C in *MT-ND6*	260,283–288
MELAS (0.18:100 000)	<20 y	Rapid progression to death after onset	HCM (most common), DCM, RCM, HT, HF, WPW, SCD	*MT-TL1* with 80% of cases attributed to m.3243A>G; m.13513G>A in *MT-TL1*, *MT-TK*	44,46,49,50,248,260,289–291
MERRF (1:100 000)	10–20 y	Progression to death within 2–15 y	DCM, HCM, WPW, SVT, RBBB, histiocytoid cardiomyopathy rare	m.8344A>G (83%–90% of cases), m.8363G>A, m.8356T>C, m.8361G>A in *MT-TK*	44,49,260,292–294
MIDD (6:100 000)	<35 y		HCM, DCM, WPW, SND, AF	m.3243A>G in *MT-TL1*	260,291,295
NARP (1:12 000–40 000)	3–12 mo		Rare; case report of peripartum CMP, HF, WPW, VT	Point sequence variations at m.8993 in *MT-ATP6* gene (most commonly m.8993T>G, then m.8993T>C)	44,260
Pearson Syndrome	Infancy	Most deaths by 3 y	LVH, LQT, depolarization abnormalities	Large mtDNA deletions ranging from 4.9 to 14 kb	296
MT-TI	Infantile and adult		HCM, DCM	m.4269A>G, m.4284G>A, m.4295A>G, m.4300A>G	297–301
MT-TL1	Infantile and adult		Heart block, DCM, HCM, syncope, SND, SCD	m.3252A>G, m.3260A>G, m.3303C>T	302–304
Other mt-tRNA genes			HCM, rare DCM		50

AF indicates atrial fibrillation; CMP, cardiomyopathy unspecified; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; HF, heart failure; HT, hypertrabeculation; LBBB, left bundle branch; LHON, Leber hereditary optic neuropathy; LQT, long QT syndrome; LVH, left ventricular hypertrophy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; MIDD, maternally inherited diabetes and deafness; mt-tRNA, mitochondrial transfer RNA; NARP, neuropathy, ataxia, and retinitis pigmentosa; RBBB, right bundle branch block; RCM, restrictive cardiomyopathy; SCD, sudden cardiac death; SND, sinoatrial node dysfunction; SVT, supraventricular tachycardia; VT, ventricular tachycardia; and WPW, Wolff-Parkinson-White syndrome.

genetic counselor or geneticist, is recommended, in addition to discussion of results.[39,40]

CARDIOMYOPATHY AND ARRHYTHMIAS IN MITOCHONDRIAL SYNDROMES

Patients with mitochondrial diseases often, but not always, exhibit cardiac involvement.[41] Cardiac manifestations are genotype- and age-specific. Mitochondrial cardiac diseases can be categorized into primary and secondary forms.[42] Primary mitochondrial disease arises from pathogenic variants in either the mitochondrial or nuclear genomes and affects OXPHOS. Primary mitochondrial cardiomyopathies typically manifest with atrioventricular conduction defects and are characterized by abnormal heart muscle structure, function, or both.[43] Cardiomyopathy and ECG abnormalities are among the most common cardiac manifestations of mitochondrial diseases, affecting 29% to 40% and 39% to 68% of patients, respectively.[44–46] Pediatric patients with cardiac manifestations of mitochondrial diseases have a low survival rate of 18%; adults generally have a better prognosis.[44,45]

Mitochondrial cardiomyopathy can present in hypertrophic, dilated, or restrictive forms, and may include features of left ventricular hypertrabeculation.[47] Hypertrophic cardiomyopathy is the most common cardiomyopathy presentation and may present as early as the antenatal period.[44,45,48,49] Obstructive hypertrophic cardiomyopathy is rare.[42,44,48,50] Hypertrophic cardiomyopathy may progress to systolic dysfunction, left ventricular decompensation, and dilation.[51] Dilated cardiomyopathy, which may arise secondarily to hypertrophic cardiomyopathy or as a primary condition, is observed less frequently in patients with mitochondrial diseases, and restrictive cardiomyopathy is rare.[44,45,48,49] Some case report evidence supports a connection between histiocytoid cardiomyopathy and mitochondrial diseases, but the extreme rarity of histiocytoid cardiomyopathy prevents us from making any definitive conclusions, and more research is needed.

Patients are most likely to present with cardiomyopathy if they have the heteroplasmic *MT-TL1* tRNA$^{Leu(UUR)}$ m.3243A>G variant; MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms), with a heteroplasmy level of 50% to 80%; MIDD (maternally inherited diabetes and deafness), with a heteroplasmy level of 10% to 40%; or a lethal pediatric disease, in which heteroplasmic levels exceed 90%, although the heteroplasmy levels for these presentations can vary markedly on the basis of mitochondrial haplogroup, tissue sampled, and patient age.[52–54] Patients with MERRF (mitochondrial encephalomyopathy with ragged-red fibers), MELAS, or MIDD should be monitored with cardiac screening every 3 to 5 years for the development of either cardiac hypertrophy or dilated cardiomyopathy.[47]

The severity of cardiac disease varies. Patients with Kearns-Sayre syndrome are predisposed to atrioventricular conduction defects, which can manifest as syncope, Adams-Stokes syndrome, or sudden death. Severe cardiac manifestations encompass heart failure (HF), ventricular tachyarrhythmia, and sudden cardiac death, particularly during metabolic crises precipitated by stressors such as febrile illness, alcohol exposure, or surgery. Patients with *PPA2* variants should be counseled to abstain from alcohol, as even minimal alcohol consumption can trigger HF and sudden cardiac arrest at various ages, with inter- and intrafamilial phenotypic variability.[55] Certain forms of dilated cardiomyopathy with hypertrabeculation, such as those caused by defects in mitochondrial ribosome subunits, *DNAJC19*, or Barth syndrome, may pose life-threatening risks early in life but may stabilize by 5 to 6 years of age in some patients.[56–59] Genetic investigations are essential, and may provide insights into tailored management strategies or potential therapies once genotype–phenotype associations are better understood.

Regular cardiac screening is essential for the early detection of abnormalities in patients with mitochondrial diseases. Most available data are derived from ECGs and echocardiograms, both of which show a high prevalence of detected cardiac abnormalities in patients with a mitochondrial disease.[46] Cardiac magnetic resonance imaging has been less extensively studied in this population but holds promise as a valuable tool providing detailed insights into cardiac function and myocardial tissue characterization, supplying essential information to support differential diagnosis. Further research is needed to better understand the potential advantages and limitations of cardiac magnetic resonance imaging in the evaluation and management of mitochondrial diseases.[60]

CLINICAL CARE AND MANAGEMENT FOR MITOCHONDRIAL DISORDERS

Guidelines for diagnosing, managing, and caring for patients with mitochondrial diseases provide specific recommendations for addressing cardiomyopathy and rhythm disorders.[61–63] Coordinated care for patients with mitochondrial myopathies should occur in medical settings with access to expertise across multiple specialties, including neurology, cardiology, arrhythmia management, pulmonology, and genetics. According to guidelines, cardiac evaluations—including physical examination, ECG, ambulatory ECG, and cardiac imaging (echocardiography or cardiac magnetic resonance)—are recommended at the time of diagnosis and periodically thereafter, even in the absence of cardiac symptoms.[61,62]

Recent clinical trials aimed at improving mitochondrial function, restoring cellular homeostasis, and reducing oxidative damage have shown promise as therapeutic avenues[64]; however, no definitive evidence supports the superiority of these targeted interventions over conventional symptom-based treatments.[65] Conventional symptom-based treatments include HF therapies, pacing, cardiac resynchronization therapy, implantable cardioverter defibrillators, or, in rare, selected cases, heart transplantation.[26,62] Further research is crucial to establish the efficacy of emerging therapeutic strategies. Along with the development and testing of novel agents in well-defined and homogeneous study populations with clinically relevant primary end points, recognizing the need for flexibility, particularly given the unique challenges posed by rare diseases such as mitochondrial disorders, is essential.

RELATION OF mtDNA VARIATION WITH CVD AND RISK FACTORS

Cardiovascular risk factors (eg, dyslipidemia, hypertension, hyperglycemia, obesity, combustible cigarette smoking and exposure) and disease have been directly linked with mtDNA damage, alterations in mtDNA copy number, and mitochondrial dysfunction in cardiovascular tissues of animal models.[66–68] In utero and adult exposures of

animal models to combustible cigarette smoke has been linked to increased mtDNA damage and deletions, oxidative stress, impairments in OXPHOS complexes, and lower mtDNA copy numbers in cardiovascular tissues.[69–72] In population-level studies, higher mtDNA damage and lower mtDNA copy number in circulating blood cells are associated with CVD risk factors, including combustible cigarette use (reviewed in reference [73]), type 2 diabetes,[74,75] and advancing age.[75]

Higher levels of heteroplasmic variants in circulating blood samples are associated with advancing age[17] and CVD[5] in humans. In heart transplants of patients with ischemic heart disease, a common 5-kb mtDNA deletion is elevated 10- to 200-fold in the explanted heart.[76] Exposure to cigarette smoke affects heteroplasmy levels and mtDNA copy number to promote the penetrance of pathogenic mtDNA variants.[77–79] Both leukocyte telomere length and levels of a 4.9-kb mtDNA deletion were reported to be independent predictors of major adverse cardiovascular events in patients with coronary artery disease—those with short leukocyte telomere length and high mtDNA deletion levels had the highest risk.[80] Whether this mtDNA deletion is part of the causal pathway, is secondary to the underlying biology, or contributes to disease risk as a bystander is unclear.

Most population-level studies to date have been restricted to evaluating the association of mitochondrial haplogroups or only a few select variants with cardiovascular risk factors and disease, rather than assessing global mtDNA variation. Mitochondrial haplogroups have been associated with hypertension,[81,82] cardiomyopathies,[22,83] atherosclerosis,[84–86] myocardial infarction,[81,87,87a] ischemic stroke,[81,83,88,89] cardiac transplant complications,[12] aortic aneurysm,[82,89a] and atrial fibrillation[90] (Supplemental Table), albeit not without controversy or contradictory reports.[86,91,92] Specific mtDNA variants have been found to be associated with both cardiovascular risk factors and disease (Table 5); however, the majority of population-level studies published to date have assessed only a handful of specific mtDNA variants with polymerase chain reaction–based or microarray technologies, often in samples of <1000 individuals.

Numerous studies have identified heteroplasmic mtDNA variants in tRNA genes associated with CVDs, including coronary artery disease, coronary heart disease, and hypertension.[93–105] The tRNA variants are thought to cause changes in tRNA structure and function that impair mitochondrial protein synthesis and, therefore, mitochondrial respiration. Correlations were found between carotid intima–media thickness and degree of carotid stenosis with the VAF of $tRNA^{Leu(UUR)}$ m.3256C>T in circulating white blood cells in age- and sex-adjusted models.[106,107] The directionality of the association of mtDNA tRNA variants with carotid intima–media thickness differs, suggesting that mtDNA variants can modulate disease susceptibility. A study focusing on genetic variation in the noncoding regulatory control region of mtDNA reported both elevated and reduced risks of stroke and myocardial infarction,[108] and another study reported positive or negative relationships between heteroplasmic mtDNA variants and CVD risk factors or coronary heart disease.[109]

MODELS FOR STUDYING mtDNA VARIATION

Mouse Models

A longstanding barrier in the mitochondrial genetics field has been the inability to edit mtDNA to generate animal and cell models. Most studies to date have leveraged existing mtDNA variants present in different strains of mice to generate xenomitochondrial, conplastic, and mitochondrial–nuclear exchange mouse models.[110–112] However, such models do not allow investigators to definitively identify the mtDNA variant driving the observed phenotypes.

mtDNA variation was shown to contribute to mitochondrial dysfunction, oxidative stress, and cardiac remodeling in mitochondrial–nuclear exchange mice that underwent volume overload (a HF model).[110] The cardiac and mitochondrial abnormalities were more pronounced in mice with the C57 mtDNA, irrespective of nuclear genetic background.[110] In addition, knockout of nuclear genome–encoded mitochondrial proteins in mouse strains with different mtDNA backgrounds indicates that the interaction of variants across the genomes affects the cardiac phenotype. For example, mice lacking *ANT1*, the mitochondrial ATP/ADP exchanger, in the presence of an *MT-ND6* p.25P>L missense variant had accelerated development of cardiomyopathy, whereas in combination with the *MT-CO1* p.421V>A variant, no effect on cardiac structure and function was noted.[10] *NNT* (which transfers NAD[H] and NADP[+] across the inner mitochondrial membrane) knockout mice develop hypertrophic cardiomyopathy in the presence of the *MT-COI* 421V>A missense variant.[10,113]

In a mouse model with cardiac-specific expression of a dominant negative variant helicase that introduces mtDNA deletions, advancing age resulted in mosaicism of cardiomyocytes for mtDNA deletions, causing cardiac arrhythmias during exercise.[114] This suggests that if even a fraction of cardiomyocytes carry heteroplasmic mtDNA deletions, the biochemical defect is sufficient to cause CVD. In addition, mice carrying 2 different mtDNAs developed pulmonary hypertension and right-sided HF, and had reduced life spans, when heteroplasmy levels were >10% compared with wild-type mice.[115] Heteroplasmy is linked to cardiomyocyte death, with similar levels of cardiomyocyte loss across the ventricles, concomitant with a decrease in OXPHOS and fatty acid oxidation proteins, and a greater reliance on glucose

Table 5. mtDNA Variants Associated With Cardiovascular Risk Factors and Disease

mtDNA variants	Genes	Phenotypes	References
m.72T>C	*MT-CR*	Lower risk of MI	108
m.73A>G	*MT-CR*	Negatively associated with MI	108
m.93A>G	*MT-CR*	Associated with higher systolic BP	305
m.195T>C	*MT-CR*	Associated with ischemic stroke with replication in a second cohort	306
m.215A>G	*MT-CR*	Associated with higher LDL level	307
m.295C>T	*MT-CR*	More frequent among patients with CAD	307
m.477T>C	*MT-CR*	Associated with PAD	308
m.3197T>C	*MT-RNR2*	Associated with higher systolic BP	305
m.3243A>G	*MT-TL1*	Higher levels associated with higher pulse wave velocity, fasting insulin levels, and all-cause and stroke-related mortality risk in the general population	309
m.3256C>T	*MT-TL1*	Associated with prevalent coronary heart disease Levels correlate with systolic BP (women only), triglycerides (men only), degree of atherosclerotic stenosis, and intima–media thickness Positively correlated with intima–media thickness	106,310,311
m.3316G>A	*MT-ND1*	Higher fasting blood glucose levels	312
m.4977DEL	*MT-ND5*, *TL2*, *TLS*, *TLH*, *ND4*, *ND4L*, *TR*, *ND3*, *TG*, *CO3*, *ATP6*, *ATP8*	Higher levels of deletion independently predictive of MACEs in adjusted models Higher levels with advancing age and highest among older patients with AF Independently predictive of MACEs and all-cause mortality	80
m.5592T>C	*MT-TA*	Overrepresented among patients with coronary heart disease	95
m.5913G>A	*MT-CO1*	Higher fasting blood glucose and systolic BP	312
m.8701A>G	*MT-ATP6*	Associated with hypertension	313
m.8706A>G	*MT-ATP6*	Associated with higher waist–hip ratio	314
m.8414C>T	*MT-ATP8*	Associated with hypertension	313
m.8896A>G	*MT-ATP6*	Associated with higher waist–hip ratio	314
m.9667A>G	*MT-CO3*	Associated with PAD	308
m.10915T>C	*MT-ND4*	Associated with PAD	308
m.11914G>A	*MT-ND4*	Associated with venous thromboembolism	308
m.12315G>A	*MT-TL2*	VAF positively correlated with carotid intima–media thickness	107,311
m.12338T>C	*MT-ND5*	Associated with ischemic stroke with replication in a second cohort	306
m.12612A>G	*MT-ND5*	More frequent among patients with CAD	88
m.12705C>T	*MT-ND5*	Associated with higher mean arterial pressure	305
m.13513G>A	*MT-ND5*	VAF negatively correlated with carotid intima–media thickness	107,311
m.14124T>C	*MT-ND5*	Associated with higher fasting insulin levels	314
m.14178T>C	*MT-ND6*	Associated with total cholesterol	307
m.14272T>C	*MT-ND6*	Associated with greater body mass index	314
m.14353T>C	*MT-ND6*	Associated with higher waist–hip ratio	314
m.14584T>C	*MT-ND6*	Associated with greater body mass index	314
m.14709G>A	*MT-TE*	Positively correlated with intima–media thickness	311
m.14846G>A	*MT-CYB*	VAF negatively correlated with intima–media thickness	311
m.15059G>A	*MT-CYB*	VAF positively correlated with carotid intima–media thickness	107
m.15924A>G	*MT-TT*	Associated with higher mean arterial pressure	305
m.16089T>C	*MT-CR*	Associated with higher triglycerides	307
m.16145G>A	*MT-CR*	Associated with circulating triglycerides, left ventricular ejection fraction, and prevalent stroke	108,307
m.16172T>C	*MT-CR*	Associated with higher mean arterial pressure	305
m.16183A>C	*MT-CR*	Associated with higher systolic BP and mean arterial pressure	305

(*Continued*)

Table 5. Continued

mtDNA variants	Genes	Phenotypes	References
m.16189T>C	*MT-CR*	More prevalent among patients with CAD and type 2 diabetes Greater frequency among patients with dilated cardiomyopathy Associated with higher mean arterial pressure Greater frequency among individuals with metabolic syndrome and associated with higher circulating triglycerides, hemoglobin A1C, and HDL	315,316
m.16223C>T	*MT-CR*	Higher levels associated with MI, augmented by the presence of cardiovascular disease risk factors, including smoking	317
m.16311T>C	*MT-CR*	Greater frequency among patients with stroke	108
m.16320T>C	*MT-CR*	Associated with higher fasting glucose levels	314
m.16356T>C	*MT-CR*	Negatively associated with MI	108

AF indicates atrial fibrillation; BP, blood pressure; CAD, coronary artery disease; LDL, low-density lipoprotein; MACE, major adverse cardiovascular event; MI, myocardial infarction; mtDNA, mitochondrial DNA; PAD, peripheral artery disease; and VAF, variant allele frequency.

metabolism in the heart.[115] Similar to the limitations of the other mouse models discussed thus far, it is not clear which of the 34 mtDNA variants that differ between the 2 laboratory strains used in the study may be driving the cardiac phenotype.

Two mouse models carrying heteroplasmic *MT-TA* variants (m.5024C>T, m.5019A>G) with differential effects on tRNA-Ala have provided new insights into the cardiovascular effects of specific heteroplasmic variants.[116,117] Although both variants are in the same tRNA-encoding gene, the cardiac transcriptome differed between mice carrying one or the other heteroplasmic variant.[117] Mice carrying the m.5024C>T variant develop mild, progressive cardiomyopathy, which can be partially rescued by increasing mtDNA levels with TFAM (transcription factor A, mitochondrial) overexpression without changing heteroplasmy levels.[118] Therefore, increased TFAM levels reduced mtDNA processing, including mtDNA transcription and replication.[119,120] Whether increased mtDNA amount, increased mtDNA half-life, and replication and transcription account for the m.5024C>T rescue remains an open question. Highly increased mtDNA levels can be highly deleterious for the heart: an elevated mtDNA replication rate does not disturb fetal development, but manifests as dramatic postnatal lethal cardiomyopathy in mice.[121] Such data and the recent literature have suggested ferroptosis as a contributing mechanism of mitochondrial-related cardiomyopathy.[121,122] More mouse models carrying variants found in patients are needed to gain insights into the mechanisms of mitochondrial diseases.

Invertebrate Models

Caenorhabditis elegans and *Drosophila* are particularly advantageous model animals to address these obstacles due to tractable genetics, ease of CRISPR (clustered regularly interspaced short palindromic repeats)/Cas-based editing, transparent tissues amenable to live microscopy, ease of husbandry, and low cost.[123] Furthermore, hundreds of wild isolates of *C elegans* and *Drosophila* subspecies have been identified, with a wide range of mtDNA variants or deletions.[124] Therefore, it may be possible to leverage genetic crosses to introduce a plethora of mtDNA variants into backcrossed laboratory strains and thus compare how the deletion or truncation of specific mtDNA-encoded genes alters mitochondrial health in a system where the nuclear genotype is controlled.[125–127] Invertebrate models enable the capture of tissue-specific mitochondrial morphology, the locations of individual mtDNA nucleoid complexes, and the recruitment of replication factors through microscopy within living animal tissue.[128,129] Although *C elegans* enable studies of the basic biology of mitochondrial genetics, they lack a cardiovascular system; *Drosophila* have a heart tube.

Cell Models

Cybrids have been the primary cellular model to study the effects of specific mtDNA variants on cellular function. Cybrids are generated by treating cancer cell lines with low levels of ethidium bromide or expression of viral proteins to deplete the cell of its mtDNA, called a rho0 cell.[130,131] The rho0 cell is then fused with the platelets or the cytoplasm of enucleated cells from an individual carrying a pathogenic mtDNA variant.[130,131]

Cybrids carrying the m.3243A>G variant, the most prevalent pathogenic mtDNA variant, have provided insights into the effect of VAF on cellular phenotype. The VAF spectrum has differential effects on cellular gene expression, with small increases in VAF having modest effects on maximal mitochondrial respiration, which remained intact until the VAF was >60%.[52] However, despite intact mitochondrial respiration, cybrids with 30% to 40% heteroplasmy for the m.3243A>G variant had coordinately lower expression of genes involved in mTOR signaling and growth pathways, likely explaining the smaller cell size observed at these VAFs.[52] Glycolytic gene expression was similar for cybrids with

the m.3243A>G variant at VAFs of 20% to 30% and cybrids lacking the m.3243A>G variant; however, when m.3243A>G was present at VAFs of 50% to 90%, the glycolytic enzymes were coordinately upregulated, which correlated with declines in complex IV levels and OXPHOS.[52] The transcriptional alterations observed by cybrids carrying different m.3243A>G heteroplasmy levels correspond with epigenetic alterations, particularly histone acetylation and methylation as a result of differential effects of heteroplasmy on cellular metabolism and the availability of epigenetic substrates.[52,53] Hence, the levels of a heteroplasmic pathogenic variant exert some differential effects on cellular phenotype, which likely contributes to the phenotypic variability in mitochondrial diseases.

Cybrids allow for the evaluation of a single mtDNA variant, but the cell types used, most frequently an osteosarcoma cell line, are not reflective of cardiovascular cell types and limit the ability to interrogate mtDNA–nuclear genome interactions, particularly in the setting of a nuclear genetic modifier that may be present in the patient from whom the platelets were derived. With new advances in stem cell biology, several groups have generated induced pluripotent stem cells (iPSCs) from patients carrying pathogenic mtDNA variants for disease modeling.[132–135] The reprogramming of somatic cells (most often fibroblasts or blood cells) to iPSCs mimics the mtDNA bottleneck that occurs during epiblast specification.[132,136] The reprogramming process can introduce new heteroplasmic mtDNA variants[137]; however, most studies indicate that heteroplasmic mtDNA variants present in the somatic cells persist in the resulting iPSCs, with subclones often obtained carrying varying levels of heteroplasmic variants, which may be amplified or negatively selected against.[132,134,138,139] Most heteroplasmic mtDNA variants in iPSCs appear to be stable with time in culture and passaging, but some variants appear to undergo selective pressures with prolonged culture time.[134,138,140] Hence, generating iPSCs with stable heteroplasmic mtDNA variants is possible, but may depend on the specific mtDNA variant. Due to the potential for dynamic changes in mtDNA variants because of reprogramming, prolonged culture, or differentiation, it is essential to conduct genetic sequencing to confirm the genotype for all iPSC lines and differentiated cell types.

Although some mtDNA pathogenic variants, primarily when present at high levels, interfere with the directed differentiation of iPSCs to cardiomyocytes, others have no effect on cardiomyocyte differentiation.[134,135,140] In the few studies that have generated cardiomyocytes from the directed differentiation of iPSCs, the levels of heteroplasmic variants in the iPSCs most often persist in the derived cardiomyocytes.[132,140] Studies have not yet fully leveraged iPSCs carrying mtDNA variants to determine the mechanisms by which specific variants alter cardiomyocyte phenotype.

mtDNA Gene Editing Approaches

Exciting developments in the past few years have led to new technologies for targeted editing of mtDNA. Initial methods developed for genetic manipulation of mtDNA leveraged the absence of mitochondrial double-stranded DNA repair. Mitochondrially targeted nucleases (eg, zinc-finger nucleases, TALE [transcription activator-like effector] nucleases) were designed to recognize the mtDNA sequence containing the pathogenic variant and induce a double-stranded DNA break, resulting in the selective degradation of mtDNA copies carrying the pathogenic variant.[141–146] However, such methods are dependent upon cells being heteroplasmic for the targeted variant and do not allow for the introduction of new mtDNA variants or editing of insertions and deletions.

More recently, TALE or zinc-finger proteins targeting deaminases to the mitochondria have been developed, but have off-target effects, particularly the introduction of bystander variants within the targeted sequencing window.[147–152] The TALE domains appear to be among the sources of off-target editing, likely due to nonspecific DNA binding and interactions with endogenous transcription factors.[147,149,152] Introducing new point sequence variations in a targeted manner continues to be a challenge, and other forms of genetic manipulation, such as transcriptional repression and editing of insertions or deletions, remain unattainable. The inability to target guide RNAs into the mitochondrial matrix due to the absence of appropriate transporters in the mitochondrial membrane prevents the implementation of CRISPR-Cas technologies for manipulating mtDNA and expression. As gene editing technologies evolve and become more precise, the ability to target the mtDNA will open new possibilities to model mitochondrial genetic variation in animal and cell models.

MODELS FOR STUDYING NUCLEAR-ENCODED OXPHOS GENES IMPLICATED IN MITOCHONDRIAL CARDIOMYOPATHY

Nuclear-encoded OXPHOS genes are also implicated in mitochondrial cardiomyopathies. Pathogenic variants in nuclear-encoded genes of OXPHOS subunits and assembly factors often result in a deficiency for the affected OXPHOS complex, suggestive of impaired assembly or stability. Few knockout models of individual OXPHOS complex subunits exist, likely because a complete knockout is lethal.[153–156] Most phenotyping of OXPHOS deficiencies is restricted to patient-derived dermal fibroblasts and either HeLa or HEK-293 cells.[157–167] Such studies indicate that the affected subunit interferes with different stages of the OXPHOS complex assembly, resulting in the accumulation of complex monomers, different complex assembly intermediates, or the absence of the complex in supercomplexes with varying degrees

of residual OXPHOS complex activity and mitochondrial respiration.[162–170]

Physiologic measures and metabolic abnormalities of OXPHOS nuclear-encoded complex deficiencies have not been deeply interrogated in cardiovascular tissues and cell types, with a few exceptions. Two mouse models of complex I deficiency have been generated: one in which *Ndufs4* was knocked out globally and a second in which *Ndufs6* was knocked down globally, both with cardiac phenotypes.[171,172] Mice with *Ndufs6* knockdown develop HF with subsequent premature death.[171] Before HF onset, the *Ndufs6* knockdown mice had reduced cardiac ATP generation and an increase in hydroxyacylcarnitines, likely due to NADH feedback inhibition of key enzymes in fatty acid oxidation.[171] In contrast, *Ndufs4* knockout mice had bradycardia and were prone to arrhythmias, likely due in part to inhibitory hyperacetylation of a sodium channel involved in regulating calcium transient decay, which was attenuated with nicotinamide riboside treatment-induced increase in NAD^+/NADH levels.[172]

Several complex IV deficiency models have been generated.[155,173] Complex IV has 2 subunits, *COX6A* and *COX7A*, both of which have a ubiquitous and a skeletal muscle/heart-specific isoform, encoded on different genes (as opposed to alternative splicing).[174] *COX7A* isoforms alter the higher-order organization of complex IV, particularly in dictating when complex IV exists as a monomer versus a dimer and its ability to be incorporated into respirasomes.[175] Knockout of the skeletal muscle and cardiac-specific *Cox7a* isoform, *Cox7a1*, in a mouse model resulted in exercise intolerance and dilated cardiomyopathy despite an upregulation of *Cox7a2* and higher cardiac ATP levels compared with wild-type mice.[155] The skeletal muscle and cardiac-specific *COX6A* isoform, *COX6A2*, regulates complex IV activity in response to ADP and ATP levels.[176] Mice lacking skeletal muscle and cardiac *Cox6a2* have impaired diastolic function despite cardiac ATP levels similar to wild-type mice. The number of mitochondria and cardiac tissue histology were similar between *Cox6a2*-deficient mice and wild-types, suggesting that altered upstream metabolic processes or cellular signaling likely underlie cardiac pathology rather than insufficient ATP, but such processes were not evaluated.[173]

MECHANISMS OF MITOCHONDRIAL GENETICS IN CVDS

Transcriptional and Translational Alterations

In mammalian cells, hundreds to thousands of copies of mtDNA are distributed throughout the mitochondrial network and are packaged into discrete, nucleoprotein complexes, termed mitochondrial nucleoids, that are distributed by cycles of mitochondrial fusion and fission.[177] Whereas the mtDNA encodes the 13 essential proteins of the respiratory chain required for energy production through OXPHOS, all of the machinery required to express those genes is encoded in the nuclear genome.[178] Exquisitely regulated mitonuclear communication is required to ensure OXPHOS biogenesis and assembly.[178,179]

The nuclear genome and mtDNA each have their own transcription and translation machinery.[180–182] Transcription factors, including PGC-1α (peroxisome proliferator–activated receptor gamma, coactivator 1-α) and PGC-β (peroxisome proliferator–activated receptor, gamma coactivator 1-β), and the nuclear respiratory factors facilitate coordinated gene expression across the genomes. Transcription and translation of the mtDNA-encoded OXPHOS subunits occur within the mitochondrial matrix, whereas the nuclear genome–encoded subunits are transcribed in the nucleus and translated in the cytosol. Whereas the nuclear-encoded OXPHOS genes are present in only 2 copies, mtDNA is present in multiple copies; however, not all mtDNA copies are transcribed, ensuring proper stoichiometry of the subunits for each OXPHOS complex.[182]

mtDNA is transcribed into a polycistronic transcript by the mitochondrial RNA polymerase, along with TFAM and TFB2M (transcription factor B2, mitochondrial), with initiation occurring at 2 strand-specific promoters in the noncoding region.[180,181] The tRNAs punctuate most mRNAs and the ribosomal RNAs, and are excised to allow translation of mtDNA-encoded transcripts to occur. mtDNA-encoded transcripts lack 5′ untranslated regions and 5′ caps, differing from nuclear genome–encoded transcripts. With the exception of *MT-ND6*, the mtDNA-encoded mRNAs are 3′ polyadenylated. Translation of the mtDNA-encoded OXPHOS transcripts is performed by the mitoribosome and uses mtDNA-encoded tRNAs, as a different coding system is used for mtDNA compared with the nuclear genome. For some OXPHOS complexes, translational activators of the mtDNA-encoded transcripts also serve as chaperones for nascent OXPHOS subunits.[182] Hence, appropriate OXPHOS complex stoichiometry is also obtained by keeping the mitochondrial translational activators bound to and sequestered by unassembled mtDNA-encoded subunits, preventing additional mitochondrial translation until nuclear-encoded subunits are imported for assembly of the complex.

In humans, mtDNA copy number and expression are higher in the heart than in less energetically demanding tissues.[183] Downregulated mitochondrial biogenesis, including mtDNA transcription, is a feature in mouse models of dilated cardiomyopathy, accompanied by increased glucose reliance.[42,184] High-throughput analyses of the murine cardiac transcriptome revealed downregulation of aminoacyl-tRNA synthetases and mitoribosome assembly factors as well, suggesting a general decline in all steps of gene expression downstream of mtDNA copy

number maintenance.[184] Deleterious sequence variations in mitochondrial tRNAs, as well as inborn errors of specific nuclear-encoded mitochondrial tRNA synthetases, consistently cause maternally inherited cardiomyopathy.[185–187] Thus, transcriptional dysregulation downstream of perturbed bidirectional signaling between mitochondria and the cell nucleus is a consistent signature of both dilated and hypertrophic cardiac disease.

Metabolic Consequences of Altered Mitochondrial Respiration

Mitochondrial function is essential for cellular functions and survival. Alterations in mitochondrial respiration affect biosynthetic activities, including the supply of fundamental products for amino acid, fatty acid, cholesterol, purine, and porphyrin synthesis.[188] In addition, through the production and regulation of various metabolites and cofactors, including acetyl-CoA, α-ketoglutarate, succinate, fumarate, 2-hydroxyglutarate, and NAD^+, the mitochondrion plays an epigenetic role in gene expression.[189–191]

Mitochondrial respiration regulates calcium uptake and storage through the action of the mitochondrial calcium uniporter and sodium/calcium exchanger, located within the mitochondrial inner membrane.[192,193] Dysregulation of both the mitochondrial calcium uniporter and sodium/calcium exchanger have been associated with CVD (HF, hypertrophy, and ischemia/reperfusion injury).[194–196] Mitochondrial membrane potential is known to regulate mitochondrial calcium uniporter and sodium/calcium exchanger activities, and reactive oxygen species production plays a key role in regulating these transporters. Thus, mtDNA variants that alter mitochondrial economy also influence membrane potential and reactive oxygen species production.[197–199] Overall, mtDNA variants and changes in heteroplasmy that affect the OXPHOS economy likely affect many functions of the organelle, but our understanding of the mechanisms are still being elucidated in the setting of cardiovascular pathophysiology.[200]

Inflammation

Mitochondria play multiple roles in metabolism, including initiation of the inflammatory response. By virtue of its bacterial origin, components of the organelle, including the mtDNA, upon release act as damage-associated molecular patterns that interact with pattern recognition receptors to initiate innate immune pathways.[200–202] mtDNA damage–associated molecular patterns interact with several innate immune receptors, including TLR9 (Toll-like receptor 9), cGAS (cytosolic cyclic GMP-AMP), and the NLRP3 inflammasome.[203–205] Binding of the mtDNA to TLR9 activates NFκB (nuclear factor kappa B) signaling and transcription of proinflammatory cytokines.[201,206–209] Binding to the inflammasome activates processing of cytokines, including IL-1β (interleukin-1β).[203–205] Activation of cGAS by the mtDNA produces cyclic dinucleotide GMP-AMP, a ligand for the stimulator of interferon genes (STING), which activates the transcription factor interferon regulatory factor 3 and the expression of interferon stimulatory genes and type I interferon response.[210–212] The release of mtDNA and activation of cGAS-STING signaling has been shown to contribute to a chronic inflammatory environment that promotes a number of CVDs in animal models, although the mechanisms are still being determined.[213]

Whereas TLR9 is expressed in immune cells, it is also expressed in endothelial cells and cardiomyocytes. mtDNA damage–associated molecular patterns and TLR9 have been implicated in vascular dysfunction in hypertensive rats[214] and preeclampsia in humans,[215,216] and are suspected to play a role in vascular disease development[203,217–221] with studies using synthesized agonists for TLR9 promoting atherogenesis in mice.[222] Studies have reported associations of cGAS-STING in atherosclerosis, musculoskeletal disorders, pulmonary injury, and several metabolic diseases; it has also been reported that exposure to CVD risk factors, such as cigarette smoke (which causes mtDNA damage[69–72,223–226]), activates the cGAS-STING pathway.[227] Oxidized mtDNA can also activate 2 inflammasomes—AIM2 (absent in melanoma 2) and NLRP3 (NOD-like receptor family pyrin domain containing 3)—that trigger inflammation and cell death.[228–231]

DISCUSSION AND FUTURE DIRECTIONS

Although mitochondrial abnormalities are known to contribute to the pathogenesis of many CVDs, the contribution of mitochondrial genetic variation is understudied. Most population-level studies have focused on the evaluation of mitochondrial haplogroups or only a handful of mtDNA variants with CVD risk factors and outcomes. The majority of mitochondrial genetic epidemiology studies have not accounted for known confounding variables, including age and smoking status; often do not adjust for multiple testing; lack external validation; and have limited sample sizes. NGS creates a new opportunity to study variants across the mtDNA in its entirety and the interactions of the mitochondrial and nuclear genomes within human populations and to improve mitochondrial disease diagnostics. With the initiatives to generate large-scale population genomics data sets, such as the National Heart, Lung, and Blood Institute Trans-Omics for Precision Medicine program, All of Us, UK Biobank, FinnGen, and others, new opportunities exist to study mitochondrial genetic variation in relation to CVD risk factors, disease, and outcomes. The large-scale population genomic data sets are bereft of ancestral diversity, with most data sets consisting of sequences primarily from individuals of

European ancestry.[232,233] Increasing the diversity of ancestries represented in population genomic data sets will be essential for health equity in mitochondrial genetics.

A longstanding barrier in the mitochondrial genetics field has been the inability to edit the mtDNA to generate animal and cell models of mitochondrial diseases or carrying specific mtDNA variants. Additional obstacles in the field include (1) little understanding of mitochondrial homeostasis, and specifically the maintenance of mtDNA integrity, within the native tissue environments; (2) a lack of studies on how mitokines, such as GDF-15 (growth differentiation factor-15) and FGF-21 (fibroblast growth factor-21), primarily released from skeletal muscle in individuals with mitochondrial myopathy, affect distal organs, including the heart; (3) an absence of studies into the mechanisms underlying the association of cardiovascular risk factors, such as hypertension, and disease with mtDNA genetic variants; and (4) a dearth of data on how mtDNA integrity or copy number may vary within the tissues of an individual compared to between individuals. Interindividual variation is crucial to inform biomarker development, which, once known, could clarify why mitochondrial disease presentation and prognosis vary so greatly between those with the same diagnosis. Use of large-scale omics, paired with machine learning approaches, could be leveraged to better classify patients with different mitochondrial disease presentations and to better inform which patients may derive benefit from novel therapeutic approaches.

CONCLUSIONS

Although limitations and barriers remain, the ability to edit mitochondrial genes in both the nuclear and mitochondrial genomes creates new possibilities for understanding the effects of individual mtDNA variants and genetic interactions between the genomes. The directed differentiation of iPSCs into cardiovascular cell types, with important caveats regarding cardiomyocyte maturity, allows for cellular modeling of mitochondrial genetic variation and the identification of mechanisms by which mitochondrial genetic variants alter cardiovascular cellular biology. NGS is accelerating the identification of pathogenic mitochondrial variants in patients, our understanding of mtDNA dynamics, and population-level studies of mtDNA variation. As bioinformatic approaches, mtDNA gene editing tools, and single-cell methods continue to evolve, so does our ability to more deeply understand and appreciate the role of mitochondrial genetics in cardiovascular biology and disease.

ARTICLE INFORMATION

The American Heart Association makes every effort to avoid any actual or potential conflicts of interest that may arise as a result of an outside relationship or a personal, professional, or business interest of a member of the writing panel. Specifically, all members of the writing group are required to complete and submit a Disclosure Questionnaire showing all such relationships that might be perceived as real or potential conflicts of interest.

This statement was approved by the American Heart Association Science Advisory and Coordinating Committee on August 13, 2025, and the American Heart Association Executive Committee October 22, 2025. A copy of the document is available at https://professional.heart.org/statements by using either "Search for Guidelines and Statements" or the "Browse by Topic" area. To purchase additional reprints, call 215-356-2721 or email Meredith.Edelman@wolterskluwer.com

The American Heart Association requests that this document be cited as follows: Fetterman JL, Chinnery PF, McClellan R, Wallace DC, Suomalainen A, Ojala T, Lewis SC, Ballinger SW; on behalf of the American Heart Association Council on Genomic and Precision Medicine; Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation; Council on Cardiovascular and Stroke Nursing; and Council on Peripheral Vascular Disease. Mitochondrial genetics in cardiovascular health and disease: a scientific statement from the American Heart Association. *Circulation*. 2025;152:e•••–e•••. doi: 10.1161/CIR.0000000000001393

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Disclosures

Writing Group Disclosures

Writing group member	Employment	Research grant	Other research support	Speakers' bureau/ honoraria	Expert witness	Ownership interest	Consultant/ advisory board	Other
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(*Continued*)

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This table represents the relationships of writing group members that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all members of the writing group are required to complete and submit. A relationship is considered to be "significant" if (a) the person receives ≥$5000 during any 12-month period, or ≥5% of the person's gross income; or (b) the person owns ≥5% of the voting stock or share of the entity, or owns ≥$5000 of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

*Modest.

†Significant.

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This table represents the relationships of reviewers that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all members of the writing group are required to complete and submit. A relationship is considered to be "significant" if (a) the person receives ≥$5000 during any 12-month period, or ≥5% of the person's gross income; or (b) the person owns ≥5% of the voting stock or share of the entity, or owns ≥$5000 of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

*Modest.

†Significant.

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