Review

Endocrine Manifestations and New Developments in Mitochondrial Disease

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Abbreviations: BMI, body mass index; FSGS, focal segmental glomerulosclerosis; GAD, glutamic acid decarboxylase; GIT, gastrointestinal tract; GSIS, glucose-stimulated insulin secretion; ICA, islet cell antibody; IVF, in vitro fertilization; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MIDD, maternally inherited diabetes and deafness; MNGIE, mitochondrial neurogastrointestinal encephalopathy; MST, metaphase spindle transfer; NGS, next-generation sequencing; OXPHOS, oxidative phosphorylation; PGD, preimplantation genetic testing; PNT, pronuclear transfer; TFAM, mitochondrial transcription factor A; WES, whole exome sequencing; WGS, whole genome sequencing.

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Abstract

Mitochondrial diseases are a group of common inherited diseases causing disruption of oxidative phosphorylation. Some patients with mitochondrial disease have endocrine manifestations, with diabetes mellitus being predominant but also include hypogonadism, hypoadrenalism, and hypoparathyroidism. There have been major developments in mitochondrial disease over the past decade that have major implications for all patients. The collection of large cohorts of patients has better defined the phenotype of mitochondrial diseases and the majority of patients with endocrine abnormalities have involvement of several other systems. This means that patients with mitochondrial disease and endocrine manifestations need specialist follow-up because some of the other manifestations, such as stroke-like episodes and cardiomyopathy, are potentially life threatening. Also, the development and follow-up of large cohorts of patients means that there are clinical guidelines for the management of patients with mitochondrial disease. There is also considerable research activity to identify novel therapies for the treatment of mitochondrial disease. The revolution in genetics, with the introduction of next-generation sequencing, has made genetic testing more available and establishing a precise genetic diagnosis is important because it will affect the risk...
for involvement for different organ systems. Establishing a genetic diagnosis is also crucial because important reproductive options have been developed that will prevent the transmission of mitochondrial disease because of mitochondrial DNA variants to the next generation.

**Key Words:** mitochondrial DNA, diabetes mellitus, MIDD, clinical management, genomic testing, reproductive options

### Graphical Abstract

**Endocrine manifestations**
- Short stature (genotypically heterogeneous)
- Hypoparathyroidism (Single, large-scale mtDNA deletion)
- Hypoadrenalism (Single, large-scale mtDNA deletion)
- Diabetes mellitus (m.3243A>G and other mtDNA variants)
- Hypogonadism & Perrault syndrome (POLG and other nuclear genes)
- Pregnancy-related complications including gestational diabetes, hypertension and preterm delivery

**Other systemic manifestations**
- Central nervous system (stroke-like episodes, epilepsy, dementia, movement disorders & cerebellar ataxia)
- Sensorineural deafness
- Eyes (CPEO, pigmented retinal changes, optic atrophy)
- Heart (cardiomyopathy, WPW & bradycardia/rhythmia)
- Liver failure
- Kidney (FSGS, tubulopathy & CKD)
- GI system (dysphagia, chronic constipation & IPO)
- Peripheral nervous system (myopathy, neuropathy, exercise intolerance & fatigue)
- Bone marrow (sideroblastic anaemia & pancytopenia)

**Genomic testing**
1. Direct mtDNA variant/ nuclear gene testing for classic syndromes
2. Mitochondrial genome/ WES/ WGS
3. Consider muscle biopsy for specific circumstances

**Management**
1. Symptomatic treatments & supportive measures
2. Surveillance for complications
3. Genetic counseling & cascade testing of other family members
4. Reproductive options

**Preclinical & translational research**
1. Disease mechanisms
2. Natural history/observational studies
3. Trials of novel therapies

### Essential Points
- The most frequent endocrine problem in adult patients with mitochondrial disease is diabetes mellitus and that this is largely because of the common m.3243A>G mtDNA pathogenic variant. This form of diabetes mellitus is often accompanied by sensorineural deafness.
- Patients with single, large-scale mtDNA deletions may develop several different endocrine phenotypes and that patients with childhood-onset severe disease should be closely monitored.
- Involvement of different nuclear genes encoding important mitochondrial proteins may lead to very specific endocrine problems—for example, primary ovarian failure in \textit{POLG} disease.
- The investigation of mitochondrial disease has been simplified with the development of next-generation sequencing and identifying a genetic diagnosis crucial for management, providing appropriate genetic advice and enrolment to clinical trials.
- For most patients with mitochondrial disease, there are no specific curative treatments currently and management of endocrine problems is similar to other patients with hormone deficiency.
- It is crucial that patients with endocrine dysfunction from mitochondrial disease are carefully monitored for other complications of mitochondrial disease—for example, the cardiac or gastrointestinal symptoms seen in patients with m.3243A>G disease.
- There are new reproductive options for patients with inherited mitochondrial DNA pathogenic variants; these options should be discussed early with patients and families.
Mitochondria play an important role in cellular function in all tissues, including those of the endocrine system. Indeed, endocrine defects such as diabetes mellitus are prevalent in certain forms of mitochondrial disease. Over the past decade, there have been major advances in our knowledge of mitochondrial disease, including advances in diagnosis, management, and prevention (1). This review will focus specifically on the role of mitochondrial abnormalities causing endocrine problems, new developments in diagnosis, emerging therapies and reproductive options, and how these advances in our understanding of mitochondrial disease are influencing patient management.

Background Nature of Mitochondrial Biology and Genetics

Mitochondria are ubiquitous organelles present in all nucleated cells in the body. Mitochondria have multiple functions within cells, including oxidative phosphorylation, fatty acid oxidation, Krebs cycle, urea cycle, gluconeogenesis, and ketogenesis (Fig. 1). They also play an important role in several other important cellular processes including (nonshivering) thermogenesis, amino acids and lipid metabolism, biosynthesis of haem and iron-sulphur clusters, calcium homeostasis, and apoptosis. Mitochondria are dynamic organelles undergoing fission and fusion depending on the metabolic state of the cell (2).

Figure 1. Mitochondrial oxidative phosphorylation (OXPHOS) system and other pathways that are commonly implicated in mitochondrial diseases. The OXPHOS system comprises complexes I to V and 2 mobile electron carriers, CoQ10 and cytochrome c. The breakdown of carbohydrate (glycolysis) and fatty acids (beta oxidation) lead to the production of acetyl-coenzyme A (CoA), which is the first substrate of the TCA cycle (also known as the citric acid cycle or Krebs cycle). NADH and FADH2 are generated through a series of enzymatic reactions in which electrons are transferred along the mitochondrial respiratory chain (complex I-IV). High-energy electrons are passed along the complexes and protons (H+) are pumped out of the matrix space, creating an electrochemical membrane potential that is used by the ATP synthase (complex V) to phosphorylate ADP and generate ATP.

The mtDNA encodes 13 protein subunits, 22 tRNAs and 2 rRNAs; there are multiple copies of mtDNA per cell, ranging from hundreds to thousands depending on the cell type. The replication, maintenance, transcription, and translation of mtDNA and mtDNA-encoded proteins are dependent on many nuclear-encoded proteins that are synthesized in the cytosol and imported into mitochondria through specific transporters (not shown). Genetic defects in nucleotide synthesis and salvage (e.g., DGUOK, TK2), mtDNA replication and maintenance (e.g., catalytic subunit [POLG] and accessory units [POLG2] of polymerase gamma and TWNK), fusion and fission machinery (e.g., MFN2, OPA1) can perturb mtDNA integrity and copy number, leading to the formation of multiple deletions and mtDNA depletion, respectively. This figure is derived from a previous published work (1).
Oxidative phosphorylation (OXPHOS) occurs in the inner mitochondrial membrane, and there are 5 multisubunit complexes that are directly involved in OXPHOS, 3 of which pump protons into the intermembrane space (complexes I, III, and IV). This generates an electrochemical gradient across the inner membrane that dissipates through complex V to generate ATP from ADP and Pi (Fig. 1). The complexes involved in OXPHOS include subunits encoded by both mitochondrial and nuclear genomes highlighting the complex genetics of mitochondria.

The mitochondrial genome is a small (16.6 kb) circular DNA (mtDNA) that contains 37 genes, 13 protein subunits of OXPHOS, 22tRNAs, and 2 rRNAs. The 13 protein subunits consist of 7 subunits of complex I, 1 subunit of complex III, 3 subunits of complex IV, and 2 subunits of complex V. The rRNA molecules enable the intramitochondrial synthesis of these subunits. The rest of the mitochondrial proteins, including all the other subunits of OXPHOS, are nuclear encoded and transferred into mitochondria after synthesis in the cytosol.

In view of the multiple functions of mitochondria, the diseases that are traditionally (and in this review) termed mitochondrial are those in which defects of OXPHOS are the primary abnormality. This therefore includes those genetic defects involving the mitochondrial genome and specific nuclear genes involved directly or indirectly in OXPHOS. There are approximately 1100 nuclear mitochondrial proteins, including those important for the many other functions of mitochondria (3). Nuclear mitochondrial proteins are also directly, and indirectly, involved in the replication and repair of mtDNA, and thus nuclear mitochondrial defects can lead to depletion or pathogenic variants of the mitochondrial genome (4). Currently, mitochondrial disease genes can be classified based on their functional roles and pathways involved (3, 5-7): (1) OXPHOS structural subunits, assembly factors and electron carriers; (2) metabolism of cofactors and vitamin; (3) metabolism of substrates including TCA cycle and fatty oxidation; (4) mtDNA replication, maintenance, transcription, RNA processing/mutation, and translation; (5) mitochondrial membrane dynamics, composition, and quality control; and (6) others (including proteins with functions that are less well-characterized). Clinically, defects of mtDNA and nuclear DNA can look very similar, which is why establishing a genetic diagnosis is crucial.

Mitochondrial DNA genetics is very different from nuclear DNA genetics (8). Mitochondrial DNA is maternally inherited and mtDNA diseases are only passed down the female line (9-11). Cells contain multiple copies of mtDNA ranging from several hundred in some cells to hundreds of thousands in an oocyte. In the presence of a pathogenic variant of the mitochondrial genome, this can affect all copies (called homoplasm) or a mixture of wild-type and mutated copies (called heteroplasm). Homoplasmic variants are an important cause of disease with well-recognized pathogenic variants, but the majority of pathogenic variants are heteroplasmic (12). Most heteroplasmic mtDNA defects are functionally recessive and thus high levels of mutated mtDNA are required before a biochemical defect in cells and the development of clinical disease. This threshold varies for different variants and indeed there may be a difference in clinical threshold for disease dependent on nuclear (13) or environmental factors (14, 15). From a clinical perspective, it is important to recognize there is often a difference between the level of heteroplasmy in different tissues with lower levels in replicating tissues (such as blood) and much higher levels in nondividing tissues (such as muscle) (12). This is important to recognize in the diagnosis of mtDNA disease (see Mitochondrial DNA genomics).

Prevalence of mitochondrial disease
Mitochondrial diseases are one of the most commonly inherited metabolic disorders, with a prevalence in the adult population of approximately 1 in 5000 of the population. Epidemiological studies have shown that approximately two-thirds of all mitochondrial disease in adults is due to mtDNA pathogenic variants (16). The prevalence of all forms of childhood-onset (<16 years of age) mitochondrial diseases has been estimated to range from 5 to 15 cases per 100 000 individuals and to be predominantly the result of pathogenic variants in nuclear genes (approximately 80%). There is considerable variation in prevalence of childhood-onset mitochondrial disease in different populations because of genetic founder variants and high consanguinity (17). Pathogenic variants in more than 300 nuclear genes cause mitochondrial disease (5), some of which are associated with endocrine abnormalities.

Clinical Features of Mitochondrial Disease—Endocrine
Disorders of the endocrine system are frequently reported in patients with specific subtypes of mitochondrial disease and defects of OXPHOS. Although diabetes is the predominant endocrine manifestation, abnormalities of other endocrine glands are also observed. Hormone deficiency (as opposed to secretory excess) is a consistent feature of mitochondrial endocrinopathies. This is not surprising because, in general, hormone synthesis and secretion are energy-dependent processes. In theory, therefore, all endocrine organs are prone to mitochondrial dysfunction. However, certain endocrine tissues seem to be particularly susceptible to mitochondrial
dysfunction, leading to an increased prevalence of specific hormone deficiencies.

**Diabetes mellitus**

Original reports describing the role of mtDNA variants in the development of human disease highlighted the impact on the neuromuscular system (18, 19). Two papers published in 1992 provided the first strong evidence of the association of diabetes mellitus with mitochondrial DNA pathogenic variants. The first described a pedigree in which there was maternal inheritance of diabetes mellitus and/or premature deafness across 3 generations because of a 10.4-kb mtDNA duplication/deletion (20). Of the 9 affected individuals, 7 had diabetes and deafness. All of the patients with diabetes were insulin treated, and 3 had experienced diabetic ketoacidosis. In the second report, 11 individuals within a large pedigree had non-insulin-dependent diabetes and deafness with a clear maternal pattern of inheritance. An A to G transition at position 3243 in the mitochondrial MT-TL1 gene was identified that cosegregated with the diabetes and deafness (21, 22). From these initial landmark reports, it was apparent that the diabetes phenotype linked to mitochondrial dysfunction was variable and spanned insulin dependence through to non-insulin-dependent diabetes.

Subsequent papers followed, with the majority focusing on the m.3243A > G variant and its association with diabetes. Based on the expanding evidence base, Maassen and Kadowaki proposed the new diabetes subtype of maternally inherited diabetes and deafness (MIDD) because of the 3243 mtDNA variant (23); this subtype was later incorporated into the World Health Organization diabetes classification. Although other pathogenic mtDNA variants have been reported in associations with mitochondrial diabetes (see Prevalence of m.3243A > G MIDD and other mtDNA variants associated with diabetes mellitus), MIDD resulting from the m.3243A > G variant is the subtype that the endocrinologist is most likely to encounter in clinical practice and is the focus of this review.

**Diabetes phenotype in MIDD**

A key objective of this review is to provide guidance as to how to identify and investigate a patient with suspected MIDD. The accumulated evidence from case series and cohort studies has identified some common themes and highlighted areas of variation. The mean age of diagnosis of diabetes is remarkably similar between studies. In a multicenter study of 54 patients with MIDD and the m.3243A > G variant (24), the mean age of diabetes diagnosis was 39 years (range, 12-67 years). A review of 31 patients with MIDD attending a national mitochondrial disease service reported a mean age of diabetes diagnosis of 38 years for the same gene variant (25). Other studies have similarly reported that diabetes generally presents in early middle age, although with a broad age range (26). Although the mean age of diagnosis is consistent within m.3243A > G MIDD, age of diabetes diagnosis is of no real value in helping to discriminate from other forms of diabetes. That is because autoimmune diabetes (type 1 and late-onset diabetes of the adult) (27), and other monogenic forms of diabetes such as maturity-onset diabetes of the young (28, 29) can present in early middle age.

The diabetes phenotype is variable and dynamic. It was estimated that 13% of m.3243A > G MIDD patients required insulin therapy from the time of diagnosis (25), whereas 45% of the remaining non-insulin-dependent patients progressed to insulin therapy over a mean period of 4.2 years. In a cross-sectional review, 41% of patients with MIDD were classified as non-insulin dependent, whereas 13% required insulin from the time of diagnosis and 8% presented in diabetic ketoacidosis (30). Both studies were based in populations of North European extraction, but this variable diabetes phenotype is not restricted to this ethnic group. In studies of Japanese pedigrees, there was evidence of insulin and non-insulin-treated diabetes within pedigrees (26, 31, 32); in 1 family, 2 patients presented with diabetic ketoacidosis, whereas other relatives were non-insulin dependent (26). In essence, the diabetes phenotype is variable both within the MIDD subtype, and within affected pedigrees.

Despite this variable diabetes phenotype, it is important to note that m.3243A > G patients with MIDD are invariably lean at presentation (23). In a French cohort of 34 patients with MIDD, the mean body mass index (BMI) was 20.2 with a range of 13.5 to 27.1 kg/m² (24). Whittaker and colleagues reported mean BMIs of 22.3 and 23.5 kg/m², respectively, in diabetic and nondiabetic m.3243A > G variant carriers (25), whereas in a Japanese study the patients with MIDD had a mean BMI of 20.4 (±3.1 SD) kg/m² (33). It is evident that irrespective of whether the patients with MIDD are insulin or non-insulin requiring, they are almost always nonobese (BMI < 30 kg/m²), which is an important consideration from a diagnostic perspective.

**Lack of autoimmunity in MIDD**

Autoimmune diabetes is characterized by elevated titers of specific autoantibodies, in particular glutamic acid decarboxylase (GAD) and islet tyrosine phosphatase 2 (34). The observation that some m.3243A > G patients with MIDD present with diabetic ketoacidosis and/or require insulin from the time of diagnosis raised the possibility of an autoimmune process in the development of diabetes. Suzuki and colleagues tested 78 patients with MIDD and
none were autoantibody positive (33). Conversely, a study of 31 Japanese patients with MIDD identified 1 patient who was strongly positive for both GAD and islet cell antibodies (ICAs), likely to be coexisting autoimmune diabetes, whereas another 12 patients were weakly positive for ICAs (35). It was postulated that the low-grade ICA positivity in the absence of GAD antibodies in MIDD might represent an autoimmune response to partial beta-cell damage secondary to mitochondrial dysfunction (36). However, supporting mechanistic evidence is lacking, and a subsequent large multicenter study of 54 patients with MIDD identified ICA positivity in just 1 patient (24). As previously surmised, there is therefore no robust evidence that MIDD is an autoimmune-driven condition (37). However, clear GAD positivity is a marker of autoimmune diabetes that would, from time to time, be expected to coexist in families with MIDD.

Potential modulators of the MIDD diabetes phenotype

The variable diabetes phenotype in patients and their families with m.3243A > G MIDD results from the interplay of different factors. Among 5 members of the same family with m.3243A > G MIDD, the level of heteroplasmy in blood was found to range from 16% to 29% (38). Analysis of tissue from a postmortem donor with the m.3243A > G variant revealed that the level of heteroplasmy differed markedly between tissues, and varied between individual pancreatic islets (see Pathophysiology of mitochondrial diabetes). It is now recognized that many hundreds of common, but functionally weak, genetic susceptibility variants predispose to type 1 and type 2 diabetes (39). The clustering of such variants within families harboring the m.3243A > G variant would be predicted to modulate the diabetes phenotypes. First, family relatives who do not carry the m.3243A > G variant might develop classical type 1 or type 2 diabetes, which would confound the maternal inheritance pattern. Second, the diabetes phenotype may be modulated in relatives with the m.3243A > G variant by the clustering of type 1 and/or type 2 diabetes variants. This has been recognized in maturity-onset diabetes of the young (40) and is likely to hold the same importance in MIDD.

Sensorineural deafness in MIDD

The m.3243A > G variant has been shown to cause symmetric sensorineural hearing impairment secondary to cochlear dysfunction (41, 42). The mean age of onset of hearing loss has been reported at 26 (43) and 34 (44) years. The hearing impairment starts with high frequency loss, and progresses steadily over time in the majority of patients (44). The level of m.3243A > G heteroplasmy was measured in tissues other than blood (almost exclusively muscle) from 40 individuals with the m.3243A > G variant, and was found to be higher in patients with hearing impairment compared with those matched for age with normal hearing (44).

Sensorineural hearing loss is a key feature of patients with mitochondrial diabetes resulting from the m.3243A > G, with a prevalence of clinically apparent hearing loss of between 86% and 98% in cross-sectional studies (24, 33). We found that hearing loss preceded the diagnosis of diabetes by an average of 6 years (25). This pattern also applied to patients with diabetes and deafness from other mitochondrial variants (25). Furthermore, patients with maternally inherited diabetes alone, without coexisting deafness and/or neuromuscular dysfunction, did not harbor the m.3243A > G variant or any other pathological variants on sequencing the entire mitochondrial genome (45).

Taking these observations together, a history of bilateral sensorineural hearing loss developing in early adulthood and before the onset of diabetes is a strong signal to investigate for MIDD.

Prevalence of m.3243A > G MIDD and other mtDNA variants associated with diabetes mellitus

An extensive and detailed review of the prevalence of MIDD resulting from the m.3243A > G variant was conducted by Murphy and colleagues (41). The summary findings were that the prevalence rates of MIDD ranged between 0.8% and 1.5% on average for unselected European and Japanese diabetic populations, respectively, increasing to an average of 5% when the screening was restricted to patients with diabetes and a personal and/or family history of deafness.

The evidence that the m.3243A > G variant causes diabetes came from studies of pedigrees that revealed cosegregation of the variant with diabetes, and the report of the high prevalence of diabetes (~40%) in patients with the variant attending a national mitochondrial clinical service (25, 46). This prevalence rate was much higher than the prevalence of the classical forms of diabetes mellitus in the background population. Diabetes mellitus has also been identified in patients with other mtDNA variants attending the same clinical service (25). The proportion (and percentage) of patients with diabetes with the following variants was: m.14709T > C: 7/13 (54%), single, large-scale mtDNA deletion: 6/55 (11%), m.8344A > G: 3/29 (10%) and multiple mtDNA deletions: 3/43 (7%). However, this was a cross-sectional analysis with no age adjustment. Using a different approach, the same group identified 29 patients who had presented with the classical MIDD phenotype, of which 21 were carriers of the m.3243A > G variant. The remaining 8 patients carried mitochondrial point variants (m.8344A > G and m.12258C > A) or single,
large-scale mtDNA deletions. In all but one, deafness preceded the diagnosis of diabetes, and subsequent targeted clinical review revealed that 6 of these patients exhibited other features of mitochondrial disease (47). Taking this information together, it is evident that other mtDNA variants are associated with diabetes and can present as the MIDD phenotype, but the m.3243A > G is the predominant genotype.

As previously reviewed (48, 49), diabetes has been reported in patients who harbor mutations in nuclear-encoded genes involved with mtDNA maintenance. However, it can be difficult to discern whether the mutations are causative or whether the diabetes is a coincidental condition. In case series of patients with mutations in RRM2B and OPA1, the prevalence of diabetes was 3.2% and 3.5%, respectively (50, 51), comparable with the prevalence of diabetes in the background population. A higher proportion (11%) of patients with mutations in POLG had diabetes (52), but unlike MIDD, these patients invariably have severe complex, multisystem disease of which diabetes is a coexisting rather than presenting feature.

Diabetes complications and MIDD

High prevalence rates of peripheral neuropathy (58%), diabetic retinopathy (62%), and nephropathy (56%) were reported in Japanese patients with MIDD resulting from diabetic retinopathy (62%), and nephropathy (56%) were reported in patients with the m.3243A > G variant (33). A subsequent study of patients with the m.3243A > G variant compared the prevalence rates between those with and without diabetes (25). Peripheral neuropathy was much more common in those with diabetes (58% vs 8%). The prevalence of renal impairment was also higher in the patients with diabetes (13% vs 0%), and these patients presented with diabetes at a significantly lower mean age compared with those with normal renal function (26 vs 38 years). Diabetic eye disease was restricted to the patients with diabetes. No correlation was found between the variant load and the development of diabetic complications. A key question is whether the presence of the m.3243A > G variant modulates the risk of developing diabetes-related complications. This was addressed in a study of 74 patients with MIDD and the m.3243A > G variant and 134 diabetic control patients without the variant matched for factors known to influence complication risk (53). After correcting for HbA1c levels and hypertension, the prevalence of diabetic eye disease was decreased and that of renal dysfunction increased in patients with the m.3243A > G variant compared with the diabetic controls. These findings led the authors to postulate that the m.3243A > G variant modulates the risk of diabetes complications independently of known risk factors. The increased prevalence of renal dysfunction in patients with diabetes with the m.3243A > G variant was in line with an earlier report in Japanese patients (54). This increased prevalence might reflect the combination of diabetic nephropathy and focal segmental glomerulosclerosis (FSGS) resulting from the m.3243A > G variant. This is supported by the observation that all 3 patients with MIDD with moderate to severe renal dysfunction had evidence of FSGS on renal biopsy (24). Whether the m.3243A > G variant directly modulates the development of diabetic renal disease remains unanswered.

Pathophysiology of mitochondrial diabetes

Mitochondrial OXPHOS represents a key step in glucose stimulus-insulin secretion coupling. Mitochondrial DNA depletion leading to decreased mitochondrial respiratory subunit expression and decreased oxidative function has been shown to impair glucose stimulated insulin secretion in murine cell lines (55). However, the development of diabetes, as a consequence of mitochondrial dysfunction, does not appear to be limited to impaired stimulus-secretion coupling. Nuclear encoded mitochondrial transcription factor A (TFAM) is required for mtDNA biogenesis and maintenance (56). A pancreatic beta-cell TFAM knockout mouse model was created that resulted in tissue-specific mitochondrial dysfunction (56). Diabetes developed from the age of 5 weeks. Pancreatic islets from young mice revealed decreased glucose-stimulated insulin secretion (GSIS), cytochrome c oxidase depletion consistent with decreased mitochondrial subunit expression, and normal beta-cell mass. Diabetes persisted with increasing age, but islets from older (39-week-old) mice showed extensive beta-cell loss. However, the remaining beta cells showed no evidence of a severe defect of OXPHOS as determined using the histochemical reaction for cytochrome c oxidase, suggesting preferential loss of those cells with greater mitochondrial dysfunction (56). TFBI and TFBIIB are involved in mtDNA translation and transcription, respectively (57, 58). Isolated pancreatic islets from knockdown mice models of these nuclear-encoded genes both exhibited decreased GSIS, with evidence of decreased beta-cell mass in the TFBIIB knockout mice (58). Taken together, these observations from animal models suggest that diabetes results from a combination of mechanisms that include decreased beta-cell mass in addition to impaired glucose stimulus insulin secretion coupling.

In the original description of the m.3243A > G variant and maternally inherited diabetes (21), it was reported that isolated skeletal muscle mitochondria from carriers of the mutation exhibited decreased mitochondrial subunit function and impaired OXPHOS. However, there are limited studies of human pancreatic tissue patients with diabetes because of the m.3243A > G variant. A decrease in beta-cell mass is a consistent finding (59, 60), with no evidence of
increased apoptosis (59). Two studies explored the level of m.3243A > G heteroplasmy in pancreatic islet tissue. Lynn and colleagues found levels 10% to 45% in pancreatic islets from a patient with non-insulin-dependent diabetes mellitus and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome, with the expected high levels in skeletal muscle and brain (61). In this patient, there was no evidence of a severe OXPHOS deficiency in beta cells. Conversely, examination of pancreatic islet tissue from a patient who had insulin-dependent diabetes identified the presence of cytochrome c oxidase deficiency in beta cells and higher levels of variant averaging 63% heteroplasmy (60). The variability in the degree of heteroplasmy between studies may reflect different stages of the disease process in line with the observations from the TFAM mouse model.

Decreased GSIS is a consistent and primary pathophysiological feature of patients with m.3243A > G MIDD (23, 31). Conversely, the insulin secretory response to arginine was found to be normal (62, 63), consistent with direct triggering of beta-cell depolarization that is downstream of mitochondrial oxidative phosphorylation.

Insulin resistance (decreased insulin sensitivity) is a complex trait with genetic and nongenetic determinants, in particular adiposity, physical activity, and glucolipotoxicity (64). Decreased whole body sensitivity has been documented in carriers of the m.3243A > G variant (65, 66), with evidence of decreased insulin stimulated glucose uptake into skeletal muscle (65) and adipose tissue (67). However, the majority of the m.3243A > G carriers had abnormal glucose tolerance compared with the normal glucose-tolerant healthy controls, and so a confounding effect of hyperglycemia on insulin sensitivity cannot be excluded.

More recently, the advancement of induced human pluripotent cell technology derived directly from patient tissues (most commonly fibroblast) offers a promising avenue of exploring disease mechanisms of pathogenic mtDNA variants and an alternative to the animal model of investigating potential therapies (68). Several research groups have successfully generated induced pluripotent cells and derived neurons using fibroblasts of patients with m.3243A > G-related MELAS and MIDD (69-72). These cell lines recapitulate the OXPHOS dysfunction, especially complex I deficiency identified in human tissues (69), and they have been used to study cell- and tissue-specific manifestations of m.3243A > G as well as the impact of different mutant heteroplasmy on cellular functions (69, 72).

In summary, impaired pancreatic beta-cell function plays a pivotal role in the development of abnormal glucose tolerance in MIDD, with a potential modulating effect of decreased whole body insulin sensitivity that may well result from secondary factors such as hyperglycemia and decreased exercise capacity.

Short stature

Short stature is commonly associated with mitochondrial diseases. Boal et al reported a cohort of 575 adult patients with mitochondrial disease of different genotypes in the United Kingdom who were significantly shorter than their peers in the general population with a mean SD for height of -0.49 (95% CI, -0.58 to -0.39) (73). About 1 in 10 of adults with mitochondrial disease had height of below 2 SD of the population mean. Among them, those who harbored the m.3243A > G variant had significantly lower final height after adjusting for multiple comparisons. Short stature has also been reported in other forms of mitochondrial disease (74-77).

There are several explanations on how mitochondrial dysfunction affects growth and final adult height. First, mitochondrial dysfunction can adversely affect fetal and placental growth (78). At birth, infants with mitochondrial disorders have been shown to be significantly lighter than their healthy comparison groups (79, 80). In particular, babies born to mothers who harbored the m.3243A > G variants have lower birth weight (mean 2779 g; 95% CI, 2529-3029) compared with other mitochondrial genotypes (mean 3313 g; 95% CI, 3170-3456) and controls (mean 3429 g; 95% CI, 3314-3545) (81). Second, growth during early childhood is influenced by the interaction of many factors that include nutrition and coexistence of chronic disease. In patients with mitochondrial disease, reduced oral intake can be due to dysphagia (82, 83), gastroparesis, and intestinal pseudo-obstruction (84). Furthermore, coexisting chronic conditions such as diabetes mellitus and renal impairment can also restrict growth potential (48, 49). Short stature is also influenced by reduced muscle mass and lack of mobility in patients with early-onset mitochondrial disease. Neuromuscular manifestations of mitochondrial disease affect skeletal integrity and longitudinal bone growth. GH deficiency has been reported as the main cause of short stature in case reports, especially children who harbored the single, large-scale mtDNA deletion and m.3243A > G variant (85-88). However, these reports need to be balanced against those in which GH deficiency was not detected (89, 90). From a clinical perspective, GH replacement should be considered in those with clear evidence of biochemical deficiency because there have been favorable treatment outcomes in some (85, 91, 92) but not all patients (93).

Hypoparathyroidism

Hypoparathyroidism has been reported in relation to Kearns-Sayre syndrome resulting from sporadic, single mtDNA deletions (94). In an older study, Harvey and Barnett studied 14 patients with Kearns-Sayre syndrome and clinical evidence of hypoparathyroidism and compared them with 212 patients without hypoparathyroidism (76,
Thyroid disease
A recent study analyzed data from the North American Mitochondrial Disease Consortium Patient Registry (99) and reported a prevalence of hypothyroidism of 6.3% based on a sample of 352 individuals with a confirmed molecular diagnosis of mitochondrial diseases. Although the definition of hypothyroidism was broad (combining clinical and biochemical based data), the authors commented that the prevalence rate of hypothyroidism was close to that for the general US background population.

Hypoadrenalism
Primary hypoadrenalism has been reported in association with mitochondrial disease. A recent review of the literature identified 14 patients with mitochondrial disease and adrenal insufficiency; of these patients, 10 harbored mtDNA deletions, 2 patients harbored recessive POLG variants, 1 harbored recessive GFER variants, and 1 carried the m.8344A > G pathogenic variant (100). When reported, adrenal antibody titers have been negative (101). Mutations in other nuclear-encoded genes can impair mitochondrial biogenesis and have been associated with adrenal insufficiency (100). Chronic and episodic electrolyte disturbance can be a feature of mitochondrial disease (102), with adrenal insufficiency being a potential cause along with other factors such as renal tubular dysfunction.

Fertility and hypogonadism
In view of the endocrine problems seen in patients with mitochondrial disease, there are potential issues with fertility (either male or female). The importance of this is difficult to evaluate for some pathogenic variants because of the relatively small numbers of patients, but the investigation of larger cohorts of patients has been invaluable in assessing the effect of the more common pathogenic variants. In a study looking at fertility among women aged 15 years or older from a large UK cohort of mitochondrial patients, the fertility rate was compared with the general population (with data obtained from the UK Office for National Statistics). Perhaps surprisingly, pathogenic mtDNA variants had no significant effect on the female fertility rate (carriers of mtDNA variants had a rate of 63.2 live births per 1000 person-years, compared with 67.2 live births per 1000 women in the general population; $P = 0.36$) (103). In addition, there was no significant difference in fertility rates between the most severely affected carriers and a comparable group of the general population. Fertility has also been explored in male patients with mitochondrial disease, and it has been proposed that mtDNA variants or nuclear mitochondrial gene variants affect sperm motility. Looking at male fertility in a clinical setting is more difficult than female fertility because of issues of paternity. However, a recent study looked at the reproductive success of men with mitochondrial disease and that of men in the general population. The reproductive success in mitochondrial patients was only 65% of the population, although many factors could contribute including severity of disease affecting attractiveness of male patients as partners, as well as direct sperm motility issues.

Although the data on populations of mitochondrial patients is helpful in terms of overall fertility, it is well recognized that pathogenic variants in certain genes can affect the functional activity of the gonads in both males and females (49). Hypogonadism is a well-recognized feature of mitochondrial disease, and both hypergonadotrophic and hypogonadotropic hypogonadism has been reported. About one-fifth of patients with single large-scale deletions of mitochondrial DNA showed evidence of clinical and/or biochemical hypergonadotropic hypogonadism, with equal prevalence between sexes (76). In a patient with Kearns-Sayre syndrome and hypopituitarism, treatment with human chorionic gonadotrophin resulted in increased testosterone levels and improvement in the secondary sexual characteristics (104), suggesting that early recognition and management may restore biochemical derangement and result in long-term clinical benefits (105). Hypergonadotropic hypogonadism has been reported in the context of mitochondrial neurogastrointestinal encephalopathy (MNGIE) (106, 107) and/or infantile onset spinocerebellar ataxia caused by recessive variants in the TWNK gene, which encodes for mtDNA helicase Twinkle (108). In a cohort of patients with leukoencephalopathies associated with variants in AARS2, all female patients developed primary or secondary amenorrhea from ovarian failure before the age of 30 years (109). One of the most
common nuclear gene-related mitochondrial disease, dominant variants in the POLG gene (110), have been linked with primary ovarian insufficiency (111) and primary testicular failure (112).

The findings of gonadic dysgenesis in males or females along with sensorineural hearing loss should raise suspicions of Perrault syndrome, a rare autosomal recessive mitochondrial disorder. In majority of patients with Perrault syndrome, their molecular genetic diagnoses remain undetermined (113, 114). To date, pathogenic variants in 6 different genes have been identified in Perrault syndrome, 5 of which encode mitochondrial related proteins—TWNK, CLPP, LARS2, HARS2, and ERAI1. CLPP encodes a mitochondrial ATP-dependent chambered protease (115), LARS2 encodes mitochondrial leucyl-tRNA synthetase (116), HARS2 encodes mitochondrial histidyl-tRNA synthetase (117), and ERAI1 encodes a mitochondrial rRNA chaperone (118). These 5 genes that disrupt the mitochondrial homeostasis by altering its protein synthesis and degradation are widely believed to have led to the Perrault syndrome phenotype (114). Although the sixth gene associated with Perrault syndrome, the HSD17B4 gene encodes a 17-beta-estradiol dehydrogenase in the peroxisomal fatty acid beta-oxidation (119). The prelingual onset of sensorineural hearing loss can be profound in early childhood and is progressive without evidence of vestibular impairment (113, 120). Gonadic dysgenesis can vary from primary amenorrhea to primary ovarian failure in females, whereas azoospermia has been reported in males (113, 114). Additional neurological features have been associated with Perrault syndrome, namely cerebellar ataxia, sensory peripheral neuropathies, intellectual disability, and leukodystrophy (114, 121, 122). Although there are fewer than 100 patients reported with a range of clinical features, under ascertainment of true prevalence is common because males without an affected sister are likely to be categorized as nonsyndromic hearing loss. Furthermore, hypogonadism may be underdiagnosed in patients with mitochondrial disease when other systemic manifestations predominate (94). Therefore, regular evaluation of the pituitary-gonadal axis hormones as well as clinical examination of these patients as part of their systemic clinical assessment will help to avoid long-term comorbidities.

**Pregnancy**

During pregnancy, there are major endocrine and physiological changes that may either precipitate a worsening of a preexisting endocrine problem or the development of a new endocrine problem such as gestational diabetes. A systematic review published in 2011 (123) identified relatively few case reports of problems during pregnancy in patients with mitochondrial disease but they noted that there were no cohort studies. More recently, there have been 2 cohort studies that have looked more specifically at the effect of m.3423A > G variant on pregnancy outcome. One of these found that pregnancies of women with the m.3423A > G variant had significantly more gestational diabetes (16% of women). More than one-third of the pregnancies in women with m.3423A > G reported high blood pressure compared with those in the comparison group to international comparative studies of 3.6% to 9.1%. Only one-half of the pregnancies in the m.3423A > G group had normal vaginal delivery, with emergency cesarean section accounting for approximately 25% of deliveries. Babies were born earlier to mothers harboring m.3423A > G, with 53.3% of them preterm (<37 weeks). In the other cohort study of patients with m.3423A > G, 25.3% had a premature delivery and 5.5% had a gestation of ≤ 32 weeks, 12% suffered from preeclampsia, and 11% developed gestational diabetes. Based on this evidence, pregnant women with the m.3423A > G variant are at increased risk of antenatal complications, and it is therefore imperative that these women are under close combined obstetric and medical supervision during pregnancy. Screening for gestational diabetes is detailed in our online resource (124) and is in line with UK National Institute for Health and Care Excellence guidance (125).

For most other genetic defects causing mitochondrial disease, the size of the cohorts has limited our ability to assess the effect of pregnancy. However, there is now good evidence that, for women with pathogenic variants of POLG, there is a high incidence of disease onset or deterioration during pregnancy that can often lead to life-threatening consequences (126).

**Clinical Features—Nonendocrine**

The clinical phenotype of mitochondrial disease is highly heterogeneous because patients can present any age group and organ involvement (17). Many mitochondrial syndromes have been reported in the literature, and such syndromic descriptions have undoubtedly aided the pattern recognition and testing for mitochondrial disease (127). However, it is increasingly recognized that most patients do not present with a classic syndrome, and the overall clinical phenotype of individuals often evolve with time. Some genetic defects are prevalent in the general population, such as m.3243A > G variant and single, large-scale mtDNA deletion (16), and endocrinologists are likely to encounter these patient groups in clinical practice, presenting with endocrine abnormalities and other multisystem features (Table 1 and Fig. 2). In this section, a general overview of different systemic manifestations is outlined.
| Inheritance                | m.3243A > G          | Single, large-scale mtDNA deletion | Surveillance and management                                                                 |
|----------------------------|----------------------|-----------------------------------|---------------------------------------------------------------------------------------------|
| Endocrine involvement      |                      |                                   | Genetic counselling<br>Reproductive options<br>Periodic assessment for diabetic complications and CVD risk factor management<br>Pharmacological treatment algorithm (Figure 4)<br>Likely multifactorial<br>Optimize nutrition<br>Consider investigating GH deficiency in patients with childhood-onset disease |
| Diabetes mellitus          | Common (~50%)        | 10%-15%                           | Pharmacological treatment algorithm (Figure 4)<br>Likely multifactorial<br>Optimize nutrition<br>Consider investigating GH deficiency in patients with childhood-onset disease |
| Short stature              | Common               | Especially in those with childhood-onset disease (KSS) | Pharmacological treatment algorithm (Figure 4)<br>Likely multifactorial<br>Optimize nutrition<br>Consider investigating GH deficiency in patients with childhood-onset disease |
| Hypoadrenalism             | Very rare            | Especially in those with childhood-onset disease (KSS) | Exclude autoimmune etiology<br>Steroid replacement<br>Calcium supplementation, vitamin D replacement, thiazide diuretics, phosphate binders |
| Hypoparathyroidism         | Very rare            | Especially in those with childhood-onset disease (KSS) | Exclude autoimmune etiology<br>Steroid replacement<br>Calcium supplementation, vitamin D replacement, thiazide diuretics, phosphate binders |
| Audiology                  | Sensorineural hearing loss | Very common                     | Audiology testing if symptomatic<br>Hearing aids<br>Consider cochlear implant for some cases (128) |
| Neurological               | Stroke-like episodes | N/A                               | Aggressive seizure treatment<br>MRI of the head, EEG<br>Details of investigation and management of stroke-like episodes are available elsewhere (129) |
|                           | ~20%                 | N/A                               | Aggressive seizure treatment<br>MRI of the head, EEG<br>Details of investigation and management of stroke-like episodes are available elsewhere (129) |
|                           | (The clinical and radiological changes are distinctive from thromboembolic stroke) | N/A                               | Aggressive seizure treatment<br>MRI of the head, EEG<br>Details of investigation and management of stroke-like episodes are available elsewhere (129) |
| Leukodystrophy             | N/A                  | Especially in those with childhood-onset disease (KSS) (130) | MRI of the head<br>Consider testing for CSF 5MTHF<br>Consider a trial of folinic acid (131)<br>Pacing for physical activities<br>Exercise is safe and beneficial (132)<br>Monitor for lactic acidemia |
| Myopathy/exercise intolerance | Common               | Common                            | Referral to oculoplastic surgeon for eyelid corrective surgery<br>Consider prism for diplopia<br>Monitor for change in visual acuity/night vision<br>May coexist with diabetic retinopathy<br>Annual ECG and echocardiogram<br>If cardiac screening is abnormal, refer to cardiologist for further assessment and treatment (eg, treatment for LVH/HCM, electrophysiology study/ablation, PPM) (134) |
| Ophthalmological           | CPEO                 | Very common                       | Referral to oculoplastic surgeon for eyelid corrective surgery<br>Consider prism for diplopia<br>Monitor for change in visual acuity/night vision<br>May coexist with diabetic retinopathy<br>Annual ECG and echocardiogram<br>If cardiac screening is abnormal, refer to cardiologist for further assessment and treatment (eg, treatment for LVH/HCM, electrophysiology study/ablation, PPM) (134) |
| Retinal changes            | Maculopathy (133); pigmentary changes | Pigmentary changes | Referral to oculoplastic surgeon for eyelid corrective surgery<br>Consider prism for diplopia<br>Monitor for change in visual acuity/night vision<br>May coexist with diabetic retinopathy<br>Annual ECG and echocardiogram<br>If cardiac screening is abnormal, refer to cardiologist for further assessment and treatment (eg, treatment for LVH/HCM, electrophysiology study/ablation, PPM) (134) |
| Cardiac                    | Common (~25%)        | Especially in those with childhood-onset disease (KSS) | Referral to oculoplastic surgeon for eyelid corrective surgery<br>Consider prism for diplopia<br>Monitor for change in visual acuity/night vision<br>May coexist with diabetic retinopathy<br>Annual ECG and echocardiogram<br>If cardiac screening is abnormal, refer to cardiologist for further assessment and treatment (eg, treatment for LVH/HCM, electrophysiology study/ablation, PPM) (134) |
|                           | Structural: LVH, HCM, heart failure<br>Electrophysiology: pre-excitation, WPW | Structural: LVH, HCM, heart failure<br>Electrophysiology: bi-Atrifascicular heart block, complete heart block | Referral to oculoplastic surgeon for eyelid corrective surgery<br>Consider prism for diplopia<br>Monitor for change in visual acuity/night vision<br>May coexist with diabetic retinopathy<br>Annual ECG and echocardiogram<br>If cardiac screening is abnormal, refer to cardiologist for further assessment and treatment (eg, treatment for LVH/HCM, electrophysiology study/ablation, PPM) (134) |
Neurological

Approximately 80% of all patients with mitochondrial disease have evidence of neurological involvement (99). Some neurological involvements are common but not specific, such as neurodevelopmental delay in children, myopathy, and exercise intolerance in adults. In contrast, some neurological findings are strongly indicative of specific mitochondrial etiology. They are the canonical feature of syndromic diagnoses, such as sequential painless visual loss in Leber hereditary optic neuropathy, developmental regression and symmetrical basal ganglia signal abnormality in Leigh syndrome, and stroke-like episodes in MELAS syndrome. More in-depth review of neurological presentations of mitochondrial disease is available elsewhere (1, 136).

Cardiac

The spectrum of cardiac involvement in mitochondrial disease includes structural heart abnormality, for instance, cardiomyopathy, Wolff-Parkinson-White syndrome caused by preexcitation accessory pathway, and variable degrees of heart block (134). Cardiac abnormalities such as asymptomatic left ventricular hypertrophy and Wolff-Parkinson-White are often insidious at the outset. They frequently form part of multisystem disease identified via the cardiac screening in common mtDNA variants such as m.3243A > G and m.8344A > G (137). Progressive conduction defects ranging from first-degree heart block to complete heart block appear to be more specifically associated with patients with single, large-scale mtDNA deletion manifesting with Kearns-Sayre syndrome. A recent retrospective study of 260 patients showed that patients with m.3243A > G and single, large-scale mtDNA deletion were associated with the highest risk of major adverse cardiac events, including heart failure, cardiac arrest, and sudden death (138). On the other hand, cardiac manifestations could be the most prominent presenting feature (ie, Senger syndrome caused by recessive AGK variants (139) and homoplasmic m.4300G > A variant (140)). Early recognition of cardiac abnormalities and therapeutic intervention is crucial because cardiac death remains the most common cause of death in adult mitochondrial disease from pathogenic mtDNA variants (141-144). However, it is important to highlight that the risk of cardiac manifestation is genotype specific. It is rare in many common nuclear gene defects linked to chronic progressive external ophthalmoplegia and mtDNA maintenance disorder in adults (145).

Gastrointestinal

Symptoms of gastrointestinal tract (GIT) dysmotility such as early satiety, lack of appetite, bloated feeling, and...
chronic constipation are relatively common in adult patients with mitochondrial disease (83). Some patients develop severe gut dysmotility, manifesting as gastroparesis and intestinal-pseudo-obstruction (146), which are potentially life-threatening if not diagnosed and managed promptly. A UK cohort study identified that intestinal-pseudo-obstruction could develop concomitantly with stroke-like episodes in 50% of m.3243A > G cases (84). MNGIE syndrome resulting from thymidine phosphorylase deficiency is an ultra-rare mitochondrial syndrome also characterized by prominent manifestations of GIT dysfunction such as cachexia, malnutrition, severe gut dysmotility, and other neurological features including chronic progressive external ophthalmoplegia, demyelinating neuropathy, and asymptomatic leukodystrophic changes identified on cranial magnetic resonance imaging (147, 148).

In hepatocerebral syndrome secondary to mtDNA depletion (4), liver disease is severe and frequently manifests with hypoglycemia, deranged liver function test, coagulopathy, and lactic acidosis in early childhood, typically during infancy. The liver disease could progress rapidly to fulminant hepatic failure requiring liver transplantation. These children frequently have progressive neurological involvements, such as generalized hypotonia, neurodevelopmental delay, and muscle weakness, some with neuropathy and intractable epilepsy. The outcomes of hepatocerebral syndrome and liver transplantation have been variable, in part, are determined by the underlying genetic defect (149, 150). For example, some patients with POLG-related Alpers disease survived the liver transplantation but succumbed to superrefractory status epilepticus (151).

Renal
Renal involvement forms a part of the clinical phenotype in some genetic defects causing mitochondrial disease (152). The examples of kidney disease are steroid-resistant nephrotic syndrome, FSGS, tubulopathy including proximal and distal renal tubular acidosis, and cystic changes with hypertension (153). In cases of m.3243A > G variant, chronic kidney disease can emerge independent of the diabetic status (154) or precede the development of diabetes (155).
Some patients with renal involvement can progress into end-stage renal failure, and anecdotal observation would suggest that their outcome with renal replacement therapy, including transplantation, is encouraging (150, 153, 155).

Other organ involvements
Although mitochondria are ubiquitous, the tissue specificity associated with certain genetic defects remains fascinating and puzzling in clinical practice. Ekbom syndrome, characterized by multiple symmetric lipomatosis, myoclonus, ataxia, and neuropathy, is strongly associated with m.8344A > G and myoclonic epilepsy and ragged red fibers syndrome but has rarely been reported in other pathogenic mtDNA and nuclear DNA variants (156), except in cases of recessive MFN2 variants (157). Sideroblastic anemia is an uncommon presentation of mitochondrial disease and has only been consistently observed in a handful of genetic defects, such as single, large-scale mtDNA deletion, PUS1 and YARS2 (158, 159).

Diagnosis
Endocrinologists play an important role in providing guidance on the investigation and management of endocrine complications related to the mitochondrial disease. On the other hand, several scenarios should prompt endocrinologists to consider whether there is primary mitochondrial etiology for “atypical” presentations of endocrine problems such as diabetes mellitus, short stature, adrenal insufficiency, and premature ovarian failure.

Diagnostic approaches for mitochondrial disease
There have been many recent advances in genetics that have greatly simplified the diagnosis of mitochondrial disease and other genetic diseases causing endocrine problems. Establishing a genetic diagnosis is crucial in view of the potential complications of mitochondrial disease and providing accurate genetic counseling. The advent of next-generation sequencing (NGS) has made the biggest change, with patients with mitochondrial disease being identified using gene panels or more recently by whole exome (WES) or whole genome sequencing (WGS) (5).

Although there has been progress with mitochondrial biomarkers in terms of detecting neurological features of mitochondrial disease, particularly neuromuscular involvement, the use of biomarkers in terms of endocrine features is still of unknown value. The 2 most widely studied biomarkers are fibroblast growth factor-21 (160) and growth differentiation factor 15 (161), but both are best for detecting muscle involvement. Other metabolomics and proteomic biomarkers are being explored at present but are not yet in clinical practice (162, 163).

Mitochondrial DNA genomics
There is considerable variability in the availability of specific genetic tests in individual countries. Screening for specific prevalent pathogenic variants such as m.3243A > G by techniques such as pyrosequencing is still practiced in some laboratories, whereas in others testing proceeds straight to amplification of mtDNA for NGS. The high depth of coverage in such sequencing provides more sensitive detection of single nucleotide mtDNA variants and accurate quantitation of mutant load. Large-scale mtDNA rearrangements are also detected but if accurate quantitation is required, alternative approaches such as quantitative PCR should be used. Some centers also extract mtDNA sequences from WES data, although this is less reliable than the other methods described because of a relatively low depth of sequencing (164, 165).

There has to be great care in testing for pathogenic mtDNA variants in blood alone. This can be due to the marked tissue segregation of some pathogenic variants (for example, single, large-scale mtDNA deletions) and the decline of heteroplasmy of specific variants in blood over time from active selection (for example, the m.3243A > G variant (13)). In addition, the m.3243A > G variant is present at low levels in blood in approximately 1 in 250 in the population, so very low levels detected on NGS may not indicate m.3243A > G disease. For m.3243A > G, the use of additional, noninvasively obtained tissues such as urinary sediment for screening mtDNA variants is encouraged (166). If mtDNA screening is uninformative in blood and urine-derived DNA, subsequent testing of muscle may be needed to fully exclude mtDNA disease (for example, single, large-scale mtDNA deletions and de novo single nucleotide variants (167)).

Nuclear DNA genomics
Pathogenic variants in more than 300 nuclear genes cause mitochondrial disease (5), although as commented previously, relatively few of these are associated with endocrine abnormalities. However, because more than 1100 mitochondrial proteins have been identified, it is highly likely that new genetic defects will be identified. In many laboratories, unbiased WES and WGS approaches have replaced the testing single genes. The diagnostic yield of WGS is usually greater than WES because of improved detection of mtDNA sequences and copy number variants, and the ability to study noncoding region. An important part of the WGS or WES pathway is the selection of the gene list to be analyzed and whether to use a clinical exome, for example. The practices vary in different countries and potentially
between laboratories, especially those with a particular research interest. In some diagnostic laboratories, there is only a narrow phenotype-defined gene list, whereas others are using lists containing all genes causing inherited disease. As with other genetic diseases, understanding whether rare variants are pathogenic remains a challenge but the widespread adoption of the American College of Medical Genetics criteria for variant classification and the development of specific algorithms are helping (1).

MIDD Diagnostic Algorithm

Diabetes is invariably an early clinical manifestation of MIDD, and as such offers the first opportunity for detection and diagnosis of mitochondrial disease. We have created an algorithm (Fig. 3) that provides an approach and guidance to the investigation of diabetic patients who may have mitochondrial diabetes. As detailed previously, MIDD usually presents in middle age and should be considered when diabetes presents before 50 years of age. Patients are invariably nonobese, although in patients with established diabetes there may have been subsequent weight gain secondary to their diabetes therapy. In line with the recommendations of an expert forum for the diagnosis of monogenic diabetes (29), it is crucially important to identify and to exclude patients who have autoimmune diabetes as they have clear and specific therapeutic and management needs.

The combination of diabetes and sensorineural deafness is a strong signal for MIDD. However, a small proportion of patients will have no hearing impairment, although they may have other neurological features of mitochondrial disease, and/or a maternal history of diabetes and deafness. It is worth noting, however, that a maternal history of diabetes alone is not a reliable signal for MIDD (45). Screening for the m.3243A > G variant is often performed using an EDTA blood sample, but the levels of heteroplasmy can be below the detection threshold in this tissue (13), so other tissues such as uroepithelial cells, or postmitotic skeletal muscle, may require analysis (1). If the m.3243A > G variant is not detected, then we would recommend discussion with the local neurogenetics team as screening for other mitochondrial DNA variants including single, large-scale mtDNA deletion may be indicated. If this is deemed unnecessary or fails to identify a causative mitochondrial DNA variant, other forms of monogenic diabetes (29) or neurogenetic disorders (77) may need to be considered (1).

**Figure 3.** Proposed diagnostic algorithm for mitochondrial diabetes. LRPCR, long-range PCR; SNHL, sensorineural hearing loss.
Management
A significant proportion of patients with mitochondrial disease are at risk of developing multisystem complications, their health care needs are complex, and frequently require access to multiple health professionals. The most challenging patients are often managed by specialist centers for mitochondrial disease (eg, the NHS Highly Specialised Service in the United Kingdom [168], mitochondrial medicine centers in the United States [169], and the European Reference Network of Neuromuscular Diseases [170]) that facilitate the delivery of multidisciplinary care together with the local primary and hospital services. These centers also offer guidelines on care (135, 171) and advice to clinicians working in other hospitals.

Management of endocrine problems
The management of hormone deficiency in relation to mitochondrial disease is broadly straightforward and involves hormone replacement in line with standard endocrine practice.

The situation is more complicated for blood glucose management in patients with mitochondrial diabetes.

Patients presenting with clinical features consistent with insulin deficiency (weight loss and ketosis) should be managed in the same way as patients with autoimmune type 1 diabetes. Importantly, they should have access to the same educational support and metabolic monitoring systems including the noninvasive continuous glucose monitoring system.

Those presenting with non-insulin-dependent diabetes should be managed in accordance with type 2 diabetes guidance, but there are a number of considerations that influence patient management and selection of oral hypoglycemic agents:

1. Increased risk of progression to insulin deficiency. For this reason, we advocate a baseline C-peptide to gauge insulin secretory reserve and education in home ketone testing.
2. Because patients with mitochondrial diabetes are at risk of lactic acidosis, especially during acute ill health, we recommend the avoidance of metformin.
3. Patients with mitochondrial diabetes respond well to sulphonylureas, with some being particularly sensitive to recurrent hypoglycemia. We advocate therefore introducing sulphonylureas at a low dose and ensure that the patient is compliant with blood glucose monitoring.
4. Most patients with mitochondrial diabetes are nonobese and often experience GIT dysfunction that can contribute to impaired nutrition (see the Gastrointestinal section).

To try to limit these problems, we would advocate dipeptidyl peptidase-4 inhibitors rather than glucagon like peptide-1 receptor analogues, although both have been reported to effective in a small case series (172).
5. Sodium-glucose transport protein 2 inhibitors have been reported to be both cardio- and reno-protective in type 2 diabetes (173), and it is assumed that these benefits extend to patients with mitochondrial diabetes, although there have been no randomized controlled trials. Nonetheless, this class of agents have been reported to improve glycemic control in mitochondrial patients (172). These agents impart an increased risk of ketoacidosis, and so education around ketone testing and sodium-glucose transport protein 2 cessation is critically important in patients with mitochondrial diabetes.

Based on these considerations, we propose a treatment algorithm for patients presenting with non-insulin-dependent mitochondrial diabetes (Fig. 4).

General management of mitochondrial disease and surveillance for systemic involvement
With a few exceptions, the principle of clinical management of mitochondrial disease is supportive (Table 1). Several emergencies are associated with mitochondrial disease, including stroke-like episodes and refractory seizures, brainstem crisis in Leigh syndrome, and multiorgan dysfunction associated with severe metabolic acidosis (1). At-risk patients, their caregivers, and clinicians should be equipped with the knowledge of recognizing these potentially life-threatening scenarios so that prompt medical treatment, such as IV anticonvulsant for stroke-like episodes and appropriate monitoring and supportive measures, can be instigated (129).

Some clinical problems such as sensorineural hearing loss, cardiac involvement, and chronic gut dysmotility are progressive in nature. Regular review following the genetic diagnosis is necessary. There are effective interventions to ameliorate these symptoms and improve quality of life. Moreover, patients with positive cardiac screening results such as left ventricular hypertrophy and conduction defect should be referred to dedicated cardiac service for further management because of the potential severe outcome. Patients with significant central nervous system involvement or neuromuscular weakness are at risk of developing respiratory insufficiency and dysphagia; therefore, assessment of respiratory and swallowing function should be considered periodically. Given the complexity and heterogeneity of clinical manifestations in mitochondrial disease, the “1 size fits all” approach is impractical. Personalized
surveillance strategies should be developed and refined based on the findings derived from natural histories of different genetic defects.

Despite lacking robust trial evidence of proven efficacy, multivitamins and cofactors supplementation, frequently referred to as “mito cocktails,” is widely promoted by some medical providers (174) and accepted by many patients and their caregivers. According to a US survey of patients with mitochondrial disease and their caregivers, around 75% of the responders took at least 4 different supplements even though similar proportion of responders reported no clinical benefits (175). The main reasons behind such empirical use of vitamin supplements and antioxidants include the perceived safety profile compared with prescribed medicines, overstated benefits in preclinical studies and case reports, and the absence of specific therapy for most forms of mitochondrial disease. However, the financial cost of “mito cocktails,” the physical burden of supplement intakes in addition to other prescribed medications among patients with swallowing difficulties or those who rely on gastros- tomy tube, and potential harms identified in mouse models (176), are frequently underestimated. On the other hand, there are specific (and rare) circumstances that long-term supplementation of vitamin and supplement are clinically indicated (177, 178), such as those with primary CoQ10 deficiency, riboflavin transporter deficiency, biotinidase deficiency, and cerebral folate deficiency.

Clinical trials and experimental treatments

There is considerable progress in finding better therapies for different forms of mitochondrial disease over the past decade (Fig. 5) (179-181). Idebenone, an analogue of CoQ10, has become the first licensed drug approved by European Medicine Agency specifically for treating visual impairment in patients with Leber hereditary optic neuropathy in Europe (182). More recently, a phase 3 randomized, controlled clinical trial involving a form of gene therapy, allotopic expression of ND4 gene, demonstrated a clinically meaningful improvement of visual function in patients who harbors the m.11778G > A variant, which accounts for > 70% of Leber hereditary optic neuropathy cases (183).

Small molecules that promote mitochondrial biogenesis or modulate the NAD+/NADH ratio are emerging as an important and generic treatment strategy for mitochondrial
myopathy, irrespective of the underlying genetic defects (179). Several novel compounds or repurposed drugs have shown some promising results in phase 2 studies (184). However, phase 2 studies in mitochondrial disease have typically been open labelled with a small patient number. It is crucial that the therapeutic efficacy is robustly confirmed in the phase 3 trials (176).

Enzymatic defects of the nucleotide metabolism, such as deficiencies of thymidine phosphorylase and thymidine kinase 2, can lead to mitochondrial DNA depletion. Thymidine phosphorylase deficiency causes systematic accumulation of nucleosides, leading to MNGIE syndrome. There are 2 main treatment strategies (185): (1) direct removal of accumulated nucleosides through either hemodialysis or peritoneal dialysis; and (2) enzymatic replacement including platelet transfusion, allogenic hematopoietic stem cell transplantation, erythrocyte-encapsulated thymidine phosphorylase, and orthotopic liver transplant. Thymidine kinase 2 deficiency causes severe mitochondrial myopathy (186), and open-labelled studies of nucleoside therapy appeared to stabilize the disease and lead to functional improvement (187).

The onset and severity of some mitochondrial DNA diseases are determined by the mutant heteroplasmy. Two mitochondrially targeted programmable nucleases, namely the mitochondrial transcription activator-like effector nucleases or mitochondrial zinc finger nucleases, have been developed to eliminate the heteroplasmy of pathogenic mtDNA variant. Both methods have demonstrated the efficacy of reducing mutant heteroplasmy in cell lines and a mitochondrial tRNA mouse model (188, 189). However, there are inherent challenges in translating laboratory techniques into human studies, especially in patients who manifest with multisystem diseases, as the efficiency of delivering nucleases to different organs is likely to be variable.

Reproductive Options

One area of mitochondrial disease that has attracted considerable attention recently is development of reproductive options for families with mitochondrial disease. It is particularly important because of the lack of curative treatment for patients and the advancements in genetics establishing a genetic diagnosis in most patients.
Broadly genetic advice is divided into 2 main groups—those patients with a nuclear gene pathogenic variant and those with involvement of the mitochondrial genome. For families with nuclear mitochondrial disease, advice will depend on the nature of the inheritance (for example, autosomal dominant or recessive) and in certain countries options such as prenatal testing and preimplantation genetic testing (PGD) are options. For families with pathogenic variants involving the mitochondrial genome, the advice is very different, reflecting the different inheritance of the mitochondrial genome.

Mitochondrial DNA is maternally inherited and goes through a genetic bottleneck in development raising several issues as regards genetic counselling. In the presence of heteroplasmy, this transmission is complicated because the bottleneck leads to the potential for extreme variation in the level of heteroplasmy between the mother with the pathogenic mtDNA variant and her offspring (Fig. 6). This is very important when we consider the reproductive options for women with pathogenic mtDNA variants. All female carriers of pathogenic mtDNA are at risk of transmitting the mtDNA variant and thus mtDNA disease to their offspring. The risk very much depends on the mtDNA variant. For example, the majority of single, large-scale mtDNA deletions are sporadic so mothers with 1 affected child may well have other clinically unaffected children. However, there is still a small risk of transmission if a patient him- or herself has an mtDNA deletion (possibly as high as 1 in 20 (190)). For patients with the m.3243A > G variant, and many other pathogenic variants (191), the risks are very different with virtually all mothers transmitting the pathogenic variant to their offspring. For mothers with homoplasmic mtDNA defects, all offspring will inherit the pathogenic variant. (192) If a patient is male, then there have been no reports of paternal transmission of mtDNA disease; therefore, they can be reassured there is no risk of transmitting the disease.

![Figure 6](https://academic.oup.com/edrv/advance-article-doi/10.1210/endrev/bnab036/6396126)

Figure 6. Mitochondrial DNA transmission. Homoplasmic variants are transmitted from mother to offspring. The mitochondrial bottleneck explains how there can be extreme divergence in the heteroplasmy between mother and offspring. There is a genetic bottleneck during development that results in different heteroplasmy in each individual oocyte. This is a major challenge when providing genetic counselling for mothers with heteroplasmic mtDNA variants because the level of heteroplasmy will determine the clinical outcome in the offspring. This figure is derived from a previous published work (1) and created with BioRender.com.
Reproductive options for women with pathogenic mtDNA variants

Prenatal diagnosis
Prenatal diagnosis involves testing the level of heteroplasmy in tissue obtained by chorionic villus sampling or amniocentesis. The level of heteroplasmy detected in the tissue is reported to reflect that seen in children (193). One of the major challenges is deciding what the risk is for a specific level of heteroplasmy for an individual variant. Where available, the results of mtDNA heteroplasmy analyses from other family members are helpful in interpreting the prenatal mtDNA test result especially if the variant is relatively rare. Prenatal diagnosis may be an option for women with low risk of transmission, for example, patients with single, large-scale mtDNA deletions (190), who want to ensure that there is no transmission. However, with any procedure there is a risk in the procedure itself (reported at around 1% loss of pregnancy), which needs to be considered (194).

Preimplantation genetic diagnosis
PGD involves the testing of embryos and then implanting an embryo with low level of heteroplasmy. PGD is certainly a good option for those women who have mtDNA variants that widely segregate in oocytes (195). In these patients, often some oocytes will have variant levels well below the level at which symptoms occur. For other heteroplasmic mtDNA variants, the challenges of PGD are much greater. This has recently been looked at in detail for the m.3243A > G variant to determine if the level in offspring can be predicted (196). The higher the mutation load in the mother, the less likely the offspring are to have levels at which disease is unlikely. It is also important to be aware that successful birth is heavily dependent on the quality of the embryo and the embryo with the lowest level of heteroplasmy might not be the best quality embryo.

Mitochondrial donation
Recently, another potential option is available for women with pathogenic variants, mitochondrial donation (also called mitochondrial replacement therapy). The rationale for developing these in vitro fertilization (IVF) techniques was due to no options being available for women with homoplasmic or high heteroplasmic mtDNA variants to have their own biologically related child, with no risk of serious disease in the offspring. Several groups pioneered the experimental techniques in embryos using metaphase spindle transfer (MST), pronuclear transfer (PNT), and polar body transfer. Most experience in human embryos is with MST and PNT (Fig. 7). MST involves the transfer of the metaphase II spindle from the oocyte with the pathogenic variant into a donor oocyte with normal mitochondria (198). Following transfer, the oocyte is then fertilized. PNT is performed immediately after fertilization with the transfer of the male and female pronuclei from the single-cell zygote with the pathogenic mtDNA variant to the donor zygote with normal mitochondria (199). Both techniques have been evaluated using human oocytes and shown that following transfer development to clinical grade embryos is feasible.

Providing a care pathway for families with pathogenic mtDNA variants
There are many challenges when considering the reproductive options for women with mtDNA variants. It is important to discuss reproductive options early with women with pathogenic mtDNA variants because fertility declines with age and the options become more limited with time. Advice should be individualized for the specific mtDNA variant (196) and it is very important to consider the mother’s health because of increased risks during pregnancy for some women (200).

Counselling women for women with pathogenic mtDNA variants should include all options including voluntary childlessness, adoption, and oocyte donation. Oocyte donation will prevent transmission of the mtDNA variant but is limited by availability of oocytes. For those women who wish to have their own biologically related child, the possible options, depending on the variant, are prenatatal testing, PGD, and mitochondrial donation (mitochondrial replacement therapy). The availability of these different options is very dependent on what is allowed in individual countries. In view of these challenges, it is important that there is careful consideration of the care pathway available. As recently highlighted in the report on Hereditable Genome Editing from the USA Academies of Science and Medicine and the Royal Society, IVF regulation is variable in different countries (201). Some countries have a highly regulated system, whereas in others, there is little or no regulation, meaning that impartial advice can be difficult to obtain. In the UK, IVF is regulated by the Human Fertilisation and Embryology Authority and, following the passing of the Mitochondrial Donation regulations through the UK Parliament, has approved mitochondrial donation for clinical under strict regulations (202).

Conclusion
A potential role of pathogenic mitochondrial gene variants in endocrine disease was first recognized in 1992, and there have been numerous publications documenting this in case reports. More recently, large
national cohort studies have given a much better insight into the true role of mitochondrial dysfunction in endocrine disease. The major endocrine manifestation of mitochondrial disease is diabetes mellitus, and this is often accompanied by sensorineural deafness in patients with the m.3243A > G pathogenic variant. Other endocrine abnormalities are less common but include hypogonadism and infertility, adrenal insufficiency, and hypoparathyroidism.

Patients may present to the endocrinologist, and making a genetic diagnosis is important because this has significant implications for disease surveillance. The majority of patients with mitochondrial disease will have involvement of other organ systems and these can have life-threatening, but often treatable, manifestations. Establishing a genetic diagnosis also has consequences in terms of transmission of the pathogenic variant to offspring. Recent developments in reproductive technologies means that, for families with mitochondrial disease, reproductive options are available.

**Figure 7.** Mitochondrial replacement therapies. (A) Metaphase II transfer. (B) Pronuclei transfer. (A) Metaphase II transfer involves removing the metaphase II spindle from the donor oocyte and then transferring the metaphase II spindle from the patient carrying the pathogenic mtDNA variant. The oocyte is subsequently fertilized by the partner's sperm. (B) Pronuclear transfer involves transferring the pronuclei, formed immediately after fertilization, from the mother's oocyte into the enucleated oocyte of the donor woman. Both metaphase II spindle transfer and pronuclear transfer result in the nuclear DNA from both parents with the mitochondria (and mtDNA) from the donor woman. This figure is modified from a previous published work (197) and created with BioRender.com.

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