

Review article

Resolving complexity in mitochondrial disease: Towards precision medicine

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ABSTRACT

Mitochondrial diseases, caused by mutations in either the nuclear or mitochondrial genomes (mtDNA), are the most common form of inherited neurometabolic disorders. They are remarkably heterogeneous, both in their clinical presentation and genetic etiology, presenting challenges for diagnosis, clinical management and elucidation of molecular mechanism. The multifaceted nature of these diseases, compounded by the unique characteristics of mitochondrial genetics, cement their space in the field of complex disease. In this review we examine the m.3243A > G variant, one of the most prevalent mitochondrial DNA mutations, using it as an exemplar to demonstrate the challenges presented by these complex disorders. Disease caused by m.3243A > G is one of the most phenotypically diverse of all mitochondrial diseases; we outline known causes of this heterogeneity including mtDNA heteroplasmy, mtDNA copy number and nuclear genetic factors. We consider the impact that this has in the clinic, discussing the personalized management of common manifestations attributed to this pathogenic mtDNA variant, including hearing impairment, diabetes mellitus, myopathy, cardiac disease, stroke-like episodes and gastrointestinal disturbances. Future research into this complex disorder must account for this heterogeneity, benefitting from the use of large patient cohorts to build upon current clinical expertise. Through multi-disciplinary collaboration, the complexities of this mitochondrial disease can be addressed with the variety of diagnostic, prognostic, and treatment approaches that are moulded to best fit the needs of each individual patient.

1. Introduction

Mitochondrial diseases are the most common form of inherited neuro-metabolic disorders, with pathogenic DNA variants in both nuclear and mitochondrial genomes affecting an estimated 1 in 4300 individuals [1,2]. They are often challenging to diagnose because of their phenotypic heterogeneity, frequently affecting multiple systems. However, the high-throughput gene-based diagnostic technology available in the last decade, along with detailed phenotyping by highly-specialized clinical services and longitudinal natural history studies have accelerated the steep rise in identification of pathogenic mutations [2–4]. One of the first and most prevalent pathogenic mitochondrial DNA (mtDNA) single nucleotide variants to be identified, the m.3243 > G *MT-TL1* gene mutation, remains a significant challenge, not only in elucidating the precise molecular mechanisms underlying pathogenicity, but also in linking these to the wide spectrum of clinical phenotypes caused by the pathogenic variant.

In this review, we will consider m.3243A > G-related mitochondrial disease as a complex disorder, outlining our current knowledge of the factors that are believed to lead to its variable phenotypic expression

(Fig. 1). We will then examine the challenges that this imposes on patients, detailing how we can tailor clinical practice, from initial assessment through to molecular diagnosis, detailed clinical assessment and management of individual phenotypes. Finally, we will consider how the complexity of this fascinating mutation directs our clinical and laboratory research and how they, in turn, have the potential to enhance the personalized clinical experience of patients.

2. Mitochondria and the mitochondrial genome

Mitochondria are present in all nucleated cells and are the principal generators of adenosine triphosphate (ATP) via oxidative phosphorylation (OXPHOS). A dynamic organelle, the mitochondrion is involved in a variety of cellular processes including differentiation, cell growth, apoptosis, nitrogen metabolism, fatty acid metabolism [4–8]. Mitochondria are responsive to the intra-cellular environment, and have the ability to remodel in response to changes in respiratory states, through mitochondrial fission and fusion [9].

Mitochondria are unique as they are under the dual control of their own mitochondrial genome (mtDNA) and that of the nuclear genome

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[10,11]. The human mitochondrial genome is a compact 16,569 base pair, double-stranded, circular molecule that is present in multiple copies per cell and encodes 13 genes, two ribosomal RNA molecules and 22 transfer RNA (tRNA) molecules [12–14]. The multicopy nature of mtDNA can give rise to heteroplasmy - the coexistence of more than one type of mtDNA within the same cell [2]. For heteroplasmic mutations, an individual would typically not show a manifestation of disease until a ‘threshold’ level of mutation has been reached [15]. Mitochondrial disease can also result from homoplasmic mutations, where the mutation is present in every copy of mtDNA [16,17]; alterations in cellular mtDNA copy number are also linked with disease [18,19]. The inheritance of mtDNA is not controlled by the same rules as the inheritance of nuclear chromosomal genes; mitochondria and mtDNA are maternally inherited [20]. Although very rare instances of bi-parental inheritance have been described [21,22], alternative explanations [23] for these results have not been excluded, therefore maternal inheritance of mtDNA remains the recognized mechanism of inheritance in humans.

3. The m.3243A > G variant

The A to G transition at position 3243 in the mitochondrial genome within *MT-TL1* (mitochondrially- encoded tRNA^{Leu(UUR)}) was initially identified as pathogenic by Goto and colleagues in 1990, within a patient cohort diagnosed with mitochondrial encephalopathy, lactic

acidosis, and stroke-like episodes – also known as MELAS syndrome [24]. Later the same year this finding was corroborated by Kobayashi et al. [25]. Subsequent studies have shown that although m.3243A > G is the most common cause of MELAS, not all individuals diagnosed with MELAS harbour this variant, and the vast majority of individuals who carry m.3243A > G do not have a clinical diagnosis of MELAS. Population studies have estimated that 140-250 individuals per 100,000 carry the m.3243A > G variant [26,27], however the point prevalence for adult clinical disease is 40-70 times lower than this at 3.5 in 100,000 [2]. This implies that a large number of m.3243A > G carriers are clinically asymptomatic or have milder symptoms not recognized as mitochondrial disease.

There have been several attempts to elucidate the pathogenic mechanism of action of the m.3243A > G variant; hypotheses have included impairment of mt-rRNA transcription termination [28,29], a decrease in the steady state levels of aminoacylated mt-tRNA^{(Leu(UUR))} [30–32], or amino acid misincorporation [31,33,34]. There is a broad consensus that m.3243A > G causes defects in mitochondrial protein synthesis and respiratory chain activity [34,35], but a full understanding has yet to be established. The use of *transmitochondrial* cybrid cells to investigate proposed mechanisms of action of the m.3243A > G variant is one reason the question of pathogenesis remains, as being highly aneuploid and genetically unstable may mean that direct *in vitro* - *in vivo* comparisons are unreliable [34].

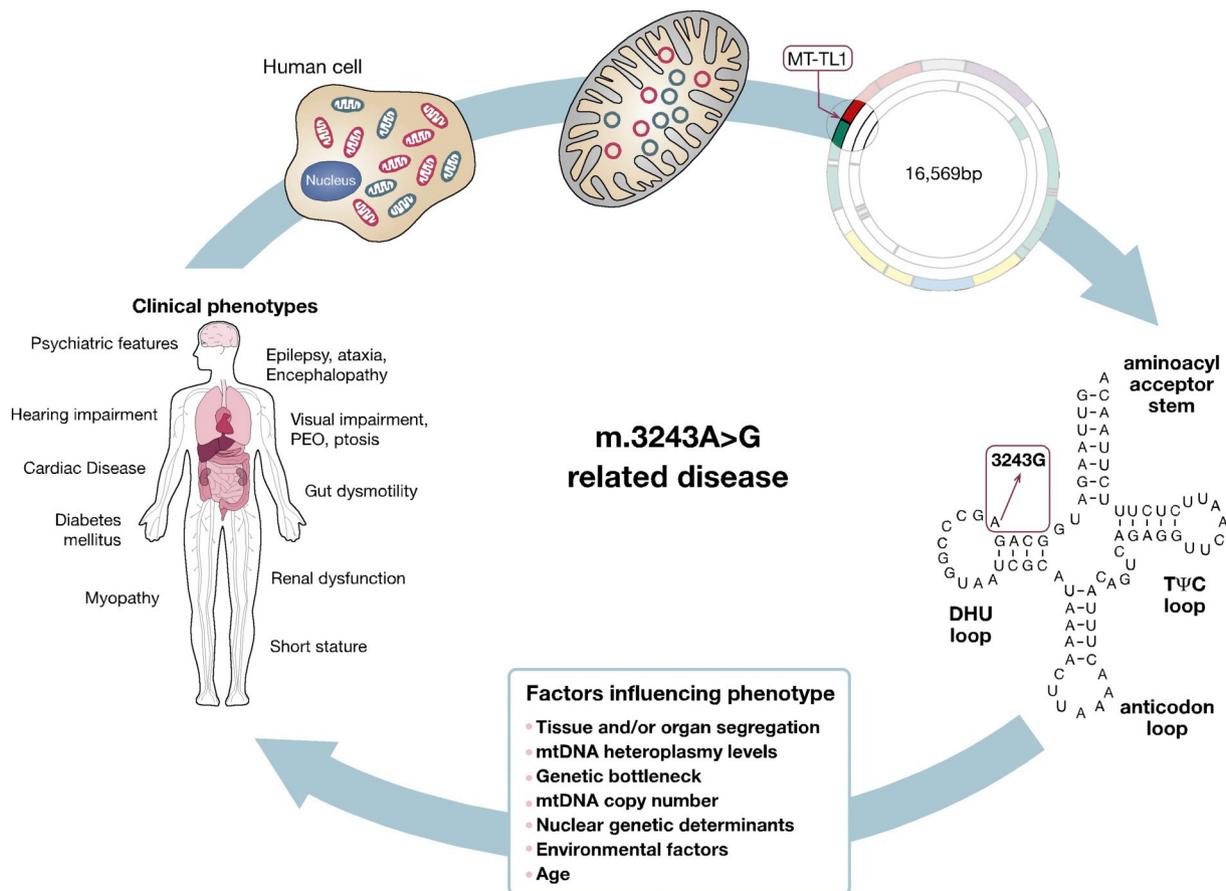


Fig. 1. Understanding m.3243A > G as a complex genetic disease. Cartoon showing how the phenotypic expression of m.3243A > G *MT-TL1* can be influenced by several factors. (Clockwise from left) The clinical phenotypes associated with this specific mtDNA variant are numerous, affecting multiple organ systems, but commonly involving the brain and CNS, auditory system, endocrine system and skeletal muscle. Human cells typically contain many mitochondria which have multiple copies of their own circular DNA. In the case of patients with the m.3243A > G variant, mtDNA is present in two forms - mutant mtDNA (shown in red) and wild-type mtDNA (shown in green). Also shown is the position of the MT-TL1 gene in the circular mitochondrial genome, and the position within the variable DHU loop of mt-tRNA^{Leu(UUR)} where the A to G substitution occurs. The phenotypic expression of this point mutation can be influenced by numerous factors as shown, including m.3243A > G mutant load (i.e. mtDNA heteroplasmy levels), the segregation of mutant mtDNAs between different organs in the body, the genetic bottleneck which governs the transmission of the m.3243A > G variant, mtDNA copy number and other factors including nuclear genetic determinants and environmental factors.

4. Phenotypic variability

The clinical presentation of m.3243A > G-related disease poses a major diagnostic challenge for clinicians; disease can occur at almost any stage of life, and can affect multiple organ systems [36]. In addition, the burden of disease and its progression is highly variable between individuals, resulting in a broad phenotypic spectrum of disease [37]. MELAS affects approximately 15% of all m.3243A > G patients [38]; of those patients diagnosed with MELAS, 80% harbor the m.3243A > G variant [39,40]. Other syndromes linked to the m.3243A > G variant include maternally inherited diabetes and deafness (MIDD) and progressive external ophthalmoplegia (PEO) [11,41]. Epidemiological studies have repeatedly shown that presentation of m.3243A > G-related disease is more frequently associated with MIDD than with MELAS [38,42–45].

A concern from epidemiological and clinical perspectives is that medical problems occurring in isolation may not be recognized as having a mitochondrial etiology and the diagnosis will be made only when a ‘syndromic’ combination of organ involvement occurs. Several of the classical syndromes related to m.3243A > G can occur in combination within a single patient and many patients demonstrate a panoply of clinical features, including myopathy, cardiac disease, short stature, kidney involvement and gastrointestinal disturbances, not consistent with any classic syndromes [38,42–47]. Another challenge is that mitochondrial disease secondary to other mitochondrial DNA or nuclear genome pathogenic mutations can present similarly to those of m.3243A > G mutation. Some of the clinical features associated with this mutation might also be evident in patients carrying other mtDNA point mutations [3,36,48].

5. Heteroplasmy

Variation in mtDNA heteroplasmy levels, the critical ratio of mutant to wild-type mtDNA molecules within the same cell, adds a degree of complexity to our understanding of m.3243A > G-related disease. Individuals who harbor the m.3243A > G variant can do so at very different levels between different cells, tissues and each other. The classic hypothesis describes a situation where biochemical deficiency is only exhibited above a tissue and mutation specific threshold of heteroplasmy. The rationale being that there is considerable redundancy in the requirement for wild type mtDNA for synthesis of mitochondrial components necessary for OXPHOS. Work in cybrid cell lines supports this hypothesis; increases in heteroplasmy levels result in abrupt transcriptional reprogramming, the mechanism of which is likely related to epigenetic changes in nuclear DNA [49,50]. However, for reasons outlined above, studies in cybrid cells must be interpreted with caution; unfortunately, demonstrating similar responses *in vivo* has proved more challenging.

Considering the threshold hypothesis, it would seem likely that the level of heteroplasmy harbored by an individual would be correlated with disease burden; this relationship is certainly not clear and is further complicated by the fact that heteroplasmy level can vary between tissues, between cells and across the lifetime of an individual [43,45,51–59]. Additionally, highly metabolic tissues that are most severely affected by m.3243A > G such as the brain and cardiac tissue, are not easily accessible in living patients and heteroplasmy measurements in autopsy samples may be influenced by necrosis caused by the disease process and so are not simple to interpret [56].

Post-mitotic tissues, such as skeletal muscle, tend to have higher and more stable levels of mutant mtDNA than more easily accessed mitotic tissues, such as blood, urinary epithelial cells and buccal mucosa [37,43,53,58,60–62]. Levels of m.3243A > G in blood fall by an estimated 1.4–2.3% per year and several methods designed to adjust blood heteroplasmy for this age-related decline have been devised [37,60,63]. Further studies are required to understand the molecular mechanism of this negative selection. Its association with mitotic cell division

indicates a process involving either a replicative disadvantage and/or a shorter life-span for stem cells carrying higher heteroplasmy levels; simulation studies support this hypothesis [63,64]. Intra-cellular selection against sub-optimal mitochondria may also contribute, but would not explain why selection does not seem to occur in post-mitotic cells.

Despite the presence of some inter-individual variation in the decline of m.3243A > G heteroplasmy in blood, recent work has shown that age-adjusted blood heteroplasmy is more highly correlated with disease burden than urinary heteroplasmy level, possibly because the latter shows much more variability within an individual. Age-adjusted blood heteroplasmy is as good a predictor as muscle heteroplasmy, although still only explains ~25% of the variability in disease burden when combined with age [37]. Individuals with similar heteroplasmy levels can present with different symptoms and some individuals with high heteroplasmy levels are relatively asymptomatic [37,38]. Additionally, the severity of some phenotypes, such as hearing impairment, diabetes, ataxia and stroke-like episodes, seem to be more influenced by high heteroplasmy than others, such as gastro-intestinal disturbance, migraine and psychiatric involvement [65], which are common comorbidities of chronic diseases in general. However, measures of age and heteroplasmy cannot be used to predict key clinical outcomes; factors other than heteroplasmy influence disease outcome in m.3243A > G carriers and may well be tissue-specific.

Heteroplasmy levels may change rapidly between generations in a phenomenon known as a mitochondrial genetic bottleneck [64,66,67]. This theory argues that only a small proportion of the total number of mitochondrial DNA molecules are transmitted to offspring from their mother. The bottleneck effect can result in mature oocytes containing different levels of heteroplasmy. The varying severity of clinical phenotypes among family members with m.3243A > G could in part be explained by this difference in the levels of transmitted mutation, however the precise mechanism of this bottleneck is yet to be defined in humans.

6. Mitochondrial DNA copy number

As well as different heteroplasmy levels, the absolute number of mitochondria and mitochondrial genomes can vary between cells, tissues and individuals. It is conceivable that the absolute wild-type mtDNA copy number is important and that higher levels could compensate for the presence of mutated mtDNA [37]. In support of this theory, a positive correlation between oxygen consumption and mtDNA copy number has been shown in m.3243A > G cybrid cells [68]. At a population level, this does not seem to be the case in less metabolically active tissues such as blood and urine, although the literature does report some conflicting results [65,69,70]. In contrast, a low skeletal muscle mtDNA copy number is associated with more severe disease. This, combined with heteroplasmy and age, increases the proportion of explained variation in disease burden to ~40% [37]. Although mtDNA copy number may simply be a marker of a patient’s level of physical activity, it is possible that cells from different individuals vary in their ability to compensate for the presence of the m.3243A > G variation and that this may influence clinical outcome [37,65,71,72].

7. Nuclear genetic modifiers

The nuclear genome encodes the majority of proteins involved in OXPHOS; their production requires the coordinated expression of nuclear and mitochondrial genes [10,73]. In fact, ~1100 nuclear-encoded proteins are believed to localize to the mitochondrion, reflecting its numerous and varied roles within the cell [74,75]. It is hypothesized that mito-nuclear cross-talk is controlled through anterograde signaling from the nucleus, however, there is some evidence to suggest that mitochondria can exert partial control over the nuclear genome, directly modifying nuclear gene expression via retrograde signaling [49,76–80]. Genetic variation may determine the way that a cell adapts to the

presence of m.3243A > G; potentially modifying metabolic pathways, mtDNA copy number and heteroplasmy. Heritable factors could also influence tissue-specific disease expression; distinct organ-specific clinical features tend to cluster in families and monozygotic twins display remarkably similar clinical phenotypes, age of phenotypic onset and tissue segregation of m.3243A > G heteroplasmy [59,81]. Therefore, genetic variation could account for differences in clinical presentation, such as the presence of diabetes, as well as the overall severity of disease.

More recently, the role of additive genetic factors in determining specific clinical phenotypes associated with m.3243A > G has been estimated across a cohort of 238 adults; psychiatric involvement, hearing impairment, ataxia, migraine and cognition display moderate to high heritability, with a larger proportion of phenotypic variance being attributable to additive genetic factors than to heteroplasmy, age and sex [65]. Interestingly, the transmission of m.3243A > G from mother to child also has a moderate heritability, implying that nuclear factors may interact with mitochondrial variants to modulate the genetic bottleneck that occurs during oogenesis [65,82,83].

It is therefore possible that the nuclear background of an individual has a greater influence on phenotypic expression than the established clinical risk factors. Moderate to high heritability values imply a strong overall genotype-phenotype correlation, but heritability does not provide any information about the genetic architecture (including the number of genetic factors involved and the relative effect sizes of each) [84]. The first step in identifying these factors is the assembly of large, well-phenotyped patient cohorts that can be used in genetic linkage and association studies [85]. Results from these studies should give us the first clues as to whether phenotypes are influenced by one or two genes with strong effects or genes acting in a polygenic fashion. Candidate nuclear genes for overall disease severity will include those with a role in oxidative phosphorylation and mtDNA replication and translation, including those previously associated with Mendelian mitochondrial diseases. For some phenotypes, such as m.3243A > G-related diabetes, causative variation may be within genes previously associated with monogenic or polygenic forms of the phenotype. In the case of diabetes, this could be genes encoding proteins within the insulin pathway or those involved in glucose sensing, such as glucokinase [86]. Identifying these nuclear factors will be challenging, but their characterization will give us further insight into the molecular mechanisms driving the phenotypic variability of m.3243A > G-related disease.

8. Other potential modifiers of mitochondrial disease development

In addition to the elements previously discussed, there are other factors known to influence the function of mitochondria. It is established that mtDNA mutations accumulate with age due to the additive effect of ROS damage in the mitochondrion, leading ultimately to the dysfunction of mitochondria [87]. The relationship between dysfunctional mitochondria and aging is not under the scope of this review, but has previously been covered in depth [87–89]. It is important to note that m.3243A > G-related disease is progressive [37,65,90], and a number of disease phenotypes are affected by age; the severity of diabetes, hearing impairment, cerebellar ataxia and neuropathy have all been strongly associated with increasing age [65]. Scoring of disease using the Newcastle Mitochondrial Disease Adult Scale has demonstrated that disease score increases with age, reaffirming the clinical observations of progressive disease [37,65,90].

Biological sex has also been proposed to alter the risk of mitochondrial disease development in patients; LHON is a mitochondrial DNA disorder where only 50% of males and 10% of females who carry a pathogenic mutation develop disease [91]; the protective effect of estrogens has been proposed to in part explain this sex bias [92]. In m.3243A > G-related disease, stroke-like episodes have been reported to occur more frequently in males than in females [45], but when

assessed in a different cohort of patients, this did not reach statistical significance [65].

A number of environmental exposures have been proposed as modifiers of disease progression in other pathogenic mitochondrial mutations. In LHON, smoking [93–95], excess alcohol consumption [94,96,97], and carbon monoxide poisoning [98], have all been reported to affect the course of disease. Exposure to antiretroviral therapy [99] has been suggested as a factor in the development of LHON and phenotypes of other mitochondrial pathogenic mutations have been suggested to be influenced by treatment with aminoglycosides [100]. In rare mutations of mitochondrial polymerase γ (POLG), treatment of individuals with valproic acid has been demonstrated to increase the risk of liver toxicity [101]. Whether these risk factors can be translated to m.3243A > G-related disease risk has not yet been confirmed.

The combination and the way in which these factors influence m.3243A > G-related disease is still not fully understood. Historically, retrospective studies have been used to disentangle the effects of multiple disease-influencing factors, but logistical problems and recall biases make it difficult for these studies to be considered reliable when employed for the investigation of rare disease [91].

9. Clinical approach to common phenotypes

It is evident that the interplay of all the factors highlighted in the aforementioned sections, including heteroplasmy levels of the m.3243A > G variant and differential tissue segregation, warrants an approach that focuses on precision medicine for these patients. Clinicians need to account for not only these genetic factors but also environmental and lifestyle elements to provide accurate diagnosis, appropriate assessment, health surveillance, counselling and effective management for this complex condition. From the outset, the emphasis of medical history-taking should be on bodily systems independently, rather than on any particular syndrome. The complexities of phenotypic expression prohibit a straightforward taxonomic approach of ascribing specific genetic mutations to a particular mitochondrial clinical syndrome. The medical history may also provide information relevant to heritable nuclear genetic factors. Carriers of the m.3243A > G variant can present with single organ disease or several overlapping features, making the diagnosis difficult for clinicians who do not encounter these regularly in their practice.

The multitude of organ involvement with high-risk of disease progression means that these patients need access to specialist services offering regular reviews with a multidisciplinary team that includes specialists from neurology, endocrinology, cardiology, physiotherapy, ophthalmology, obstetrics, clinical genetics and audiology. One model of care is to deliver specialized services for these patients through a small number of expert centers. This model is established in several European countries and is gaining favor in the United States with the recent designation of Mitochondrial Care Network with certified centers of excellence [102].

The use of validated clinical rating scales [46,103], the Newcastle Mitochondrial Disease Adult Scale or the Newcastle Paediatric Mitochondrial Disease Scale, can capture not only all the essential clinical spectrum of mitochondrial disease but also provide a semi-quantitative scoring of disease burden and a profile of disease progression, if used serially over time. Another crucial aspect of the history-taking process is family pedigree tracing. This is particularly pertinent to identify at-risk individuals due to the maternal inheritance of m.3243A > G. Clinicians should not only determine the presence of the m.3243A > G variant in the pedigree, but also the levels of mutation because heteroplasmy levels among family members may vary.

From the diagnostic perspective, the m.3243A > G variant can be sought non-invasively from either blood or urine samples. Given that heteroplasmy levels may differ in different tissues, having at least two samples in adult patients is helpful in prognostic counselling and in planning the relevant health surveillance. Heteroplasmy measurements

in a non-invasive-tissue such as blood are useful and provide a good correlation with disease burden when adjusted for age, but in cases where m.3243A > G is undetectable in blood, a second tissue such as urine is also useful to test. Skeletal muscle biopsy has historically been the ‘gold standard’ in the diagnostic work-up of mitochondrial disease because this post-mitotic tissue is relatively easily accessible and contains a large number of mitochondria for histochemical or enzyme activity analysis. Another reason that skeletal muscle tissue has been routinely used to study pathogenic mtDNA variants is that the levels of mtDNA heteroplasmy appear to correlate with the histochemical changes observed in the muscle fibers [104,105]. However, the increasing use of molecular genetic tests has diminished the need for these muscle biopsies, especially in common point mutations like m.3243A > G [106]. Although NGS can be used for determination of mtDNA heteroplasmy and deletions in mitochondrial diseases, pyrosequencing and long-range PCR are still commonly used for patients who have a suspected m.3243A > G point mutation.

There is an increasing recognition of clinical manifestations of m.3243A > G that are more common than the classical syndromes [38,44,45,47,107,108]. In the next few sections, we will summarize some of the common clinical manifestations.

9.1. Hearing impairment

Hearing impairment has been consistently the most prevalent feature reported in the literature, either as isolated manifestation or as part of a disease spectrum, with the frequency ranging from 48% to 77% [38,45,47,65,107,109–111]. The loss of hearing in carriers of m.3243A > G is significantly faster than that of presbycusis, a hearing disorder associated with ageing. Audiometry testing among carriers of the m.3243A > G mutation, which is characterized by patonal curve with downward sloping at high frequencies, has longitudinally demonstrated a loss of 2.9 dB per year for men and 1.5 dB per year for women [112,113]. Several environmental factors have been suggested as precipitants for the progression of hearing impairment [113–115], but none of them have been credibly proven. Despite being identified in population and patient cohort studies as the most prevalent feature of m.3243A > G, the precise pathophysiological mechanism of hearing loss caused by this particular point mutation has not been convincingly elucidated. Some postulate that the degenerative effect of impaired oxidative phosphorylation and of insufficient elimination of free radicals on high-energy demand structures in the inner ear as possible explanation but these hypotheses have not been tested [114,116,117]. Hearing levels for patients carrying the m.3243A > G mutation typically revealed with absent transient evoked otoacoustic emissions but preserved auditory brainstem responses, indicating that the loss is cochlear in origin [118,119]. Hearing loss has a negative impact on quality of life and it is often overlooked in patients. Therefore, clinicians should actively seek out hearing impairment in these patients, not only because of how prevalent it is but also this mildly insidious manifestation could be easily identified and corrected, albeit to a lesser degree in severe cases, with the use of hearing aids or cochlear implants.

9.2. Diabetes mellitus

Alongside hearing impairment, diabetes mellitus has been studied extensively, in part because of the prevalence of the m.3243A > G mutation within the population and its strong association with maternally inherited deafness and diabetes (MIDD) syndrome [1,2,120]. Diabetes is also common in its own right with a prevalence of 5% in the UK population. Epidemiological studies reported the frequency of the m.3243A > G mutation in unselected groups of diabetic patients ranged from 0% to 2.8% [120–123]. Given this relative scarcity of mitochondrial diabetes, in busy general diabetic clinics it can be challenging even for the most astute clinicians to consider the m.3243A > G

mutation in a patient presenting with diabetes mellitus as an isolated finding. However, atypical features for the general diabetes mellitus population, such as maternal inheritance pattern, short stature or presenile sensorineural hearing loss is likely to raise the clinical suspicion of mitochondrial disease. Another distinguishing observation from typical Type 2 diabetes mellitus is the tendency for these mitochondrial diabetic patients to have lower than average BMI [123,124]. Lower BMI in these patients paradoxically correlates with earlier onset of diabetes, earlier insulin requirements and higher HbA1c measurements [124]. The age of onset can be variable but typically develops in midlife with a median age of onset of 38 years [123,124]. Mitochondrial diabetes might develop insidiously with the gradual loss of metabolically-active pancreatic B-cells but usually transitions rapidly to insulin dependence within 2 to 4.2 years of initial diagnosis [123–125]. The risk of developing microvascular end-organ damage from the progression of m.3243A > G-related mitochondrial diabetes in the forms of peripheral neuropathy and end-stage renal disease are higher than the average Type 1 or Type 2 diabetes mellitus. Despite the rapid progression, most patients initially present with non-insulin dependent diabetes. The agent of choice in the management of these patients is sulphonylurea [120]. The risk of sulphonylurea-induced hypoglycaemia in some patients can be minimized by opting for shorter half-life preparation and by titrating slowly from the lowest dose. Another first-line agent in the general diabetic population is metformin. Although metformin has been generally avoided in patients of m.3243A > G due to the potential risk of exacerbating lactic acidosis, clinical problems are rarely observed.

9.3. Myopathy

Muscle symptoms of m.3243A > G mutation, including proximal limb weakness, exercise intolerance, extraocular muscle weakness, eyelid ptosis, muscle fatigue and pain, are common. The reported frequencies of myopathy in natural history studies and meta-analysis of m.3243A > G carriers ranged from 25% to 60% [126,127,129]. The muscle manifestations in these patients are mainly mild to moderate proximal limb weakness, especially of pelvic muscles, which result in waddling gait and modified Gower’s sign [109,126,129,130]. The increasing use of molecular genetic tests has diminished the need for the previous ‘gold standard’ muscle biopsy for diagnosis of mitochondrial disease, especially in common point mutations such as m.3243A > G [106]. The presence of cytochrome c oxidase (COX)-negative fibers, red-ragged fibers (RRF) and succinate dehydrogenase (SDH) staining has long been the defining feature of mitochondrial dysfunction in the muscle but may be absent in m.3243A > G patients. The risk of developing significant muscle symptoms in association with the m.3243A > G variant increase with age. The myopathic symptom severity has been shown to correlate with the biochemical hallmark of mitochondrial dysfunction, namely the number of COX-deficient fibers and subsarcolemmal aggregates detectable in muscle [126,130,131]. There is currently no convincing evidence for an effective treatment for mitochondrial myopathy but the increasing number of ongoing clinical trials currently provides optimism for potential disease-modifying compounds in the coming years.

9.4. Cardiac disease

Apart from skeletal muscle, the myocardium is another tissue that has a high aerobic metabolic demand and is often affected in patients with mitochondrial disease. Cardiac muscle involvement, frequently manifesting as hypertrophic cardiomyopathy, may occur in 20% to 40% of patients with m.3243A > G-related mitochondrial disease [132] and has been shown to lead to premature death [133]. Although left ventricular hypertrophy is the most dominant cardiac abnormality [134,135], more subtle cardiomyopathic changes and cardiac bioenergetic abnormalities, have been found in asymptomatic patients with normal cardiac screening [132,133,136]. Cardiomyopathy is an

independent predictor of both mortality and morbidity in these patients [132,137,138]. In addition to the involvement of cardiac muscle, rhythm abnormalities have also been reported frequently in patients with m.3243A > G including Wolff–Parkinson–White syndrome, supraventricular tachycardia, atrial fibrillation, and depolarization abnormalities [134,139,140]. In some cardiac arrhythmias, the cardiac fiber disarray could be the underlying pathology to ventricular tachyarrhythmias in some forms of hypertrophic cardiomyopathy [141,142]. Clinical reports have suggested there is a higher risk of sudden and unexpected death in asymptomatic individuals harboring the m.3243A > G mutation; thus, all carriers should have a cardiovascular risk assessment and surveillance should be according to clinical recommended guidelines [138].

9.5. Stroke-like episodes

Since the acronym MELAS was first coined, stroke-like episodes have been synonymous with m.3243A > G mitochondrial disease. The term “stroke-like” for this focal cerebral metabolic crisis is clinically misleading as the process bears very little resemblance to the strokes of arteriosclerotic or thrombo-embolic etiology. The stroke-like episode in mitochondrial disease is characterized by acute or sub-acute metabolic neurological dysfunction and, in contrast to vascular stroke, a typical onset before the fourth decade (although older onset cases have been reported) [143–145]. The prodromal features such as gradual evolution of headache and prominent visual disturbance can develop days or weeks before the onset of focal neurological deficit or motor seizure. These visual symptoms, have been described as migrainous visual aura by patients presenting for the first time, but in fact these phenomena indicate the onset of occipital epileptic seizure [146]. The severity of neurological sequelae of this cerebral metabolic crisis depends on the extent of parietal, temporal and occipital lobe involvement such as encephalopathy, dysphasia, dyspraxia, hemianopia, cortical blindness, mild hemiparesis and psychosis. Not all of these features are uniformly present. Therefore, the variable clinical picture of these “stroke-like” episode in terms of onset of symptoms, severity, frequency, duration and recovery phase can present challenges to clinicians managing patients presenting for the very first time without a mitochondrial genetic diagnosis.

An important finding on cranial neuroimaging that helps distinguish stroke-like episodes from arterial ischemic infarcts is the extension of lesions beyond anatomical vascular territories. Although these metabolic lesions have a predilection for the temporal and occipital lobes, bilateral and asymmetrical changes are not infrequent as these existing lesions evolve and predispose to further seizures [147,148]. Even if there may be no clear lesion on neuroimaging, seizures are frequently detected by electroencephalography in patients who have had a ‘stroke-like’ episode [149]. The underlying pathophysiological process associated with these ‘metabolic’ seizures has been proposed to be initiated by interneuron mitochondrial respiratory chain deficiency, which consequently alters the balance of excitation and inhibition in neural networks, promoting neuronal hyperexcitability [150]. The dramatic downregulation of OXPHOS subunits of complexes I and IV within the GABA-ergic interneurons of these patients makes these interneurons particularly vulnerable to the propagation of neuronal hyperexcitability [151]. This acute process leads to rapid neuronal damage that has been demonstrated by histopathological findings of micro-vacuolation, neuronal cell dropout, eosinophilia, astrogliosis and secondary myelin loss in patients with m.3243A > G [106]. While stroke-like episodes initially result in discreet focal neurological deficits of the hindbrain, repeated episodes impact interictal cognitive (frontal lobe) function with patients demonstrating a persistent encephalopathy and radiological evidence of brain atrophy. Psychiatric disturbance in the form of depression (rather than psychosis) is also a frequent sequel of stroke-like episodes and appears to be generally more common in mitochondrial disease patients. There are currently no established treatments

with proven efficacy in stroke-like episodes of mitochondrial origin. The existing clinical drug studies to explore definitive disease-modifying treatment have been limited by small sample size and the lack of natural history data. The difficulty in defining what constitutes a stroke-like episode and the tendency for variable degrees of spontaneous recovery means that the true effect of acute therapies is difficult to prove without large clinical randomized placebo-controlled trials.

9.6. Gastrointestinal features

Gastrointestinal symptoms due to gut dysmotility are also prevalent in the m.3243A > G patients with up to more than three quarters of carriers reporting gastrointestinal disturbance in large case-series [65]. These gastrointestinal disturbances include hard stools, flatulence and bloating [152]. The underlying pathology behind gut dysmotility in m.3243A > G patients is postulated to be a visceral myopathy, given COX-deficient smooth muscle cells harbor very high levels of m.3243A > G mutant load [153]. Pseudo-obstruction, a potentially fatal complication of bowel dysmotility, is more common in patients with high disease burden and multi-organ involvement and may occur concomitantly with, or as a heralding symptom of, a stroke-like episode [154] [155]. In busy acute general medical settings, pseudo-obstruction is an important but challenging condition to recognize. A misdiagnosis that leads to surgical intervention might increase the risk of deterioration in these vulnerable patients with high disease burden. Prompt recognition and expert medical gastroenterology input is key to avoid unnecessary surgical interventions. The risk of pseudo-obstruction can be mitigated with early detection during routine clinic surveillance for symptoms of dysphagia, constipation, abdominal pain and distention, as well as the timely management of malnutrition which is linked with the severity of gastrointestinal symptom severity [152,154,156].

The m.3243A > G mutation also has a negative impact on linear growth and weight gain; patients with more severe disease tend to be shorter with a lower BMI [157]. Our clinical observation suggests that those with early disease onset tend not to achieve their expected adult height. Low final adult height has been shown to be an indicator of disease severity and rate of disease progression [157]; thus, height and weight measurements in clinical settings are helpful for prognostication and counselling. Although m.3243A > G heteroplasmy level is negatively associated with BMI and height, the correlation is not strong; there can be marked disparities in growth and BMI between two individuals with precisely the same blood and muscle heteroplasmy, again indicating nuclear modifiers of disease expression. Other common associated symptoms with suboptimal growth are early satiety and gut dysmotility [153,158]. Our clinical experience suggests that even with the use of percutaneous gastrostomy or nasogastric tube feeding it is still very challenging to achieve satisfactory improvement in linear growth or BMI in these individuals. Nonetheless, supplementary feeding that supports good nutritional status and avoidance of further weight loss are simple measures that may improve long-term survival.

10. Prevention of transmission

The pathogenic variant m.3243A > G is maternally-inherited and so male carriers will not transmit the variant to their children, while females are highly likely to transmit at least some of the m.3243A > G variant to all of their offspring. However, the calculation of recurrence risk for women who carry the m.3243A > G is not straightforward given the unpredictable transmission, which is largely determined by the size of the genetic bottleneck. This means that mature oocytes may contain vastly different levels of m.3243A > G heteroplasmy, resulting in a highly variable risk of transmission of disease and making genetic counselling and reproductive options extremely challenging. At the moment, there are essentially four options available for at-risk couples – prenatal testing, preimplantation genetic diagnosis (PGD), oocyte donation and mitochondrial donation [159]. The first two options are

suitable for women who harbor low levels of heteroplasmy while women with high levels may benefit from the latter two options [160]. Recently, maternal m.3243A > G heteroplasmy measurements were used to calculate the risk of having a clinically affected child, which will allow evaluation of women who will benefit from mitochondrial donation [83].

11. Research in the era of precision and personalized medicine

There is a significant need to develop a deeper understanding of m.3243A > G-related disease; the vast constellations of phenotypes with which patients present is indicative that more precise definitions of disease are needed. To this end, researchers must utilize multiple sophisticated techniques to understand the variation observed on a clinical/phenotypic level, and at the genetic/cellular level. Established influences on phenotypic expression such as heteroplasmy, have already been discussed in this review, the challenge that now faces researchers is the identification of novel influencing factors and how they interact with established m.3243A > G-related disease modifiers.

The complexity of this disease is compounded by many factors, including the inter-individual variation that is present within a disease population. There can be a significant difference in presentation between the phenotypes of individuals, even when known influential factors are relatively similar. It is essential that population level differences are accounted for when approaching research of any complex disease. Effective exploration and untangling of potential genetic or environmental influences of disease requires a large cohort of patients and, ideally, family data, including information regarding asymptomatic carriers of the m.3243A > G variant. The correlation of phenotypes and genotypes of disease will facilitate the identification of new genotypes related to specific phenotypes of m.3243A > G-related disease and will make it possible to provide patients with a more precise estimation of disease burden and progression.

Clearly illustrated by the unique aspects of mitochondrial genetics, there are also variables that occur within the individual. For example, tissue-specific segregation of mtDNA means that heteroplasmy is not necessarily at a uniform level throughout the systems of the body. Accounting for intra-individual variation is a challenge that must be met in the field of complex disease research. Using a variety of longitudinally collected patient data is key to illuminating the presence of inter-individual variation; collection of patient tissue biopsies enable the elucidation of tissue specific effects, as well as calculation of heteroplasmy level, copy number and other factors which may have an impact on clinical outcome and cellular biochemical phenotype. The occurrence of inter-individual differences must also be addressed when defining the mechanism of disease. Use of multiple genetic backgrounds in cellular studies will go some way to making results more robust and translational.

Longitudinal phenotypic studies, in combination with prospective recruitment of individuals to well-defined cohorts of patients has led to groups of mitochondrial patients who are ready to participate in clinical trials. One possible route is the repurposing of already existing medications and treatments, which can significantly reduce the time it takes to be approved for patient use. Currently, there are a wide range of approaches being studied in Phase 1, 2 and 3 clinical trials in m.3243A > G patients with mitochondrial myopathy, including the targeting of 1) mitochondrial biogenesis (Bezafibrate, Nrf2 activator, Acipimox, Nicotinamide riboside); 2) oxidative phosphorylation (Coenzyme Q10, idebenone, riboflavin, thiamine); reactive oxygen species (KH176, idebenone); 3) increasing NAD⁺ (acipimox, nicotinamide riboside) and 4) cardiolipin stabilization (elamipretide) [161–164]. It is important that clinical trial cohorts are as homogenous as possible, not just those with a relevant mitochondrial mutation, but also with phenotypes that are relevant to drug targets.

12. Conclusion

The extensive phenotypic variability associated with this mitochondrial DNA variant and the unique characteristics of mitochondrial genetics, which include maternal inheritance and the multicopy nature of the mitochondrial genome, require us to treat m.3243A > G-related disease as a truly complex disorder, both in the research laboratory and the clinic. Investigating it as such, through the collaborative efforts of clinicians and researchers, will continue to empower the creation of clinical and diagnostic tools to aid with disease diagnosis, prognosis, and the prediction of an individual's response to treatment, as seen in fields such as cancer research. In turn, this will allow the development of more precise therapeutic strategies and interventions that can be applied on a patient-by-patient basis, and facilitate the bespoke tailoring of clinical care. The landscape of clinical care for patients with m.3243A > G has evolved, with increasing recognition of the range of phenotypes that can result from this single point mutation. To focus purely on the historical classification of syndromes such MELAS or MIDD is a blinkered approach to the modern day medicine for these complex patients. Advances in mitochondrial genetic sequencing have aided the diagnosis of patients without typical disease patterns or syndromes, and also allow heteroplasmy level to be assessed. Once a molecular diagnosis is made, input from several specialties is crucial in providing comprehensive and individualized care for these patients. There is currently no cure for m.3243A > G mitochondrial disease but reproductive techniques, such as mitochondrial donation, offer hope for these individuals who wish to prevent the transmission of this disorder to their offspring. As the present pursuit for disease-modifying drugs for m.3243A > G through clinical trials intensifies, it will be an exciting time for the clinical practice of mitochondrial medicine in the coming years.

Competing interests

None to declare.

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