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Review

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Renal function and mitochondrial cytopathy (MC): more questions than answers?

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Summary

Our knowledge of mitochondrial biology has advanced significantly in the last 10 years. The effects of mitochondrial dysfunction or cytopathy (MC) on the heart and neuromuscular system are well known, and its involvement in the pathophysiology of several common clinical disorders such as diabetes, hyperlipidaemia and hypertension, is just beginning to emerge; however, its contribution to renal disease has received much less attention, and the available literature raises some interesting questions: Why do children with MC commonly present with a renal phenotype that is often quite different from adults? How does a mutation in mitochondrial DNA (mtDNA) lead to disease at the cellular level, and how can a single mtDNA point mutation result in such a variety of renal- and non-renal phenotypes in isolation or combined? Why are some regions of the nephron seemingly more sensitive to mitochondrial dysfunction and damage by mitochondrial toxins? Perhaps most important of all, what can be done to diagnose and treat MC, now and in the future?

In this review we summarize our current understanding of the relationship between mitochondrial biology, renal physiology and clinical nephrology, in an attempt to try to answer some of these questions. Although MC is usually considered a rare defect, it is almost certainly under-diagnosed. A greater awareness and understanding of kidney involvement in MC might lead to new treatment strategies for diseases in which mitochondrial dysfunction is secondary to toxic or ischaemic injury, rather than to an underlying genetic mutation.

Introduction

Mitochondria are intracellular organelles present in almost all cells. They probably evolved from a primitive aerobic prokaryotic structure that fused with a larger anaerobic cell to form a new organism with a significant metabolic advantage.[1] Over time, a complex symbiotic relationship has developed between the mitochondrion and its host cell, going much further than the simple provision of ATP. Mitochondria play a central role in the regulation of a range of important cellular functions, including the generation of reactive oxygen species (ROS), intracellular Ca^{2+} homeostasis, cell proliferation and apoptosis.[2] Thus, mitochondrial dysfunction can be deleterious to the host cell in a variety of ways. Diseases associated with mitochondrial dysfunction include diabetes,[3] septic shock[4] and neuro-degenerative conditions such as Parkinson's disease.[5]

Mitochondria contain their own DNA i.e. mitochondrial DNA (mtDNA) about 16.6 kb long, which is primarily maternally inherited and encodes for 13 proteins involved in the respiratory

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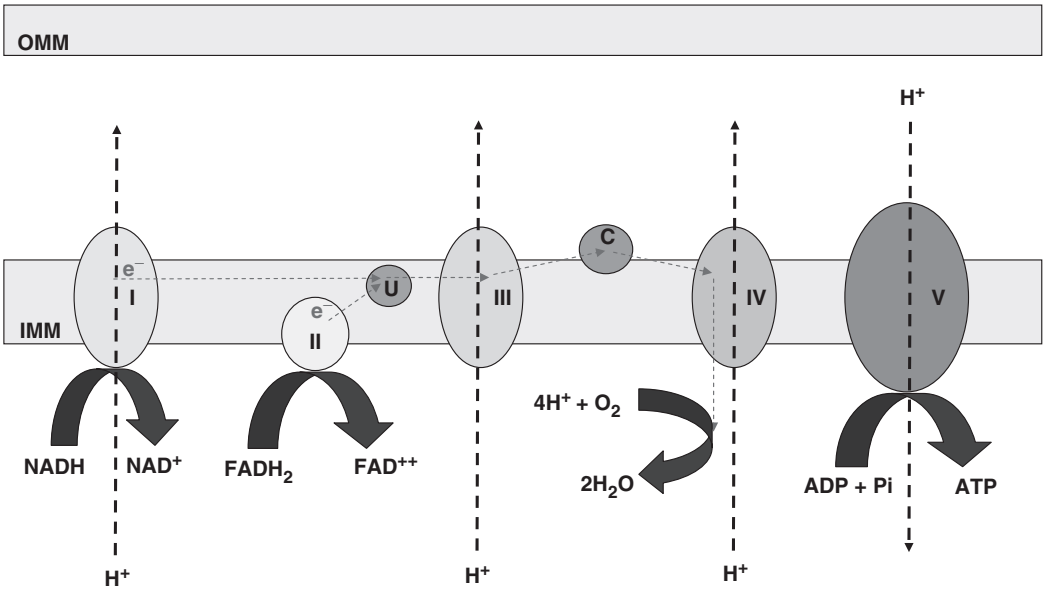

Figure 1. Electrons enter the mitochondrial RC via the oxidation of substrates NADH (complex I) and $FADH_2$ (complex II). They are then shuttled to complex III by ubiquinone (U), and then onto complex IV by cytochrome c (C). The energy released from the transfer of electrons is utilized to pump protons out of the mitochondrial matrix and into the inter-membrane space, which lies between the inner (IMM) and outer (OMM) mitochondrial membranes. Protons then pass back through complex V, down the electro-chemical gradient, powering the energetically unfavourable generation of ATP from ADP and inorganic phosphate. MtDNA encodes for subunits of complexes I, III, IV and V. Complex II is formed entirely of nuclear encoded sub-units.

chain (RC) (Fig. 1), and two rRNA subunits and 22 tRNA molecules necessary for protein synthesis. The majority of a mitochondrion's ~1000 proteins are encoded by nuclear DNA (nDNA) rather than mtDNA. Unlike nDNA, mtDNA exists as many copies in each cell and is thought to have a mutation rate as much as 10 times higher than nDNA.[6] This causes variation among cells in their mutant load, a phenomenon known as *heteroplasmy*; cells in which all copies of mtDNA are affected are said to be *homoplasmic*. With heteroplasmy, a 'threshold level' of mutant load may be necessary before cellular function is impaired enough to produce clinical disease.

Mutations in mtDNA can take the form of deletions or point substitutions. Multiple mtDNA deletions suggest a defect in nDNA, causing a secondary abnormality in mtDNA replication or repair. Diseases that are thought to be primarily due to a defect in mitochondrial function are referred to as mitochondrial cytopathy (MC), to distinguish them from conditions in which mitochondrial involvement is a secondary phenomenon, e.g. in apoptosis following ischaemia-reperfusion injury.

Paediatric renal disease due to MC

Much of the current literature on MC and renal disease comes from paediatrics and it is has been presented in several earlier published reviews.[7–9] The commonest renal manifestation seems to be the renal Fanconi Syndrome (FS);[10,11] although the nephrotic syndrome, tubulo-interstitial disease, a Bartter's-like syndrome and renal tubular acidosis have all been described.[12] So far no clear pattern has emerged linking particular gene mutations with specific RC defects or a particular renal phenotype. However, it has been noted that most of the children described present at an early age (generally under 2 years old) and renal dysfunction is diagnosed after developing a severe and generalized multi-system disorder.

A range of underlying gene defects has been described, including point mutations and deletions of mtDNA. Children with FS often have mtDNA deletions and syndromes known to be caused by mtDNA deletion, such as Pearson (refractory sideroblastic anaemia, diabetes and lactic acidosis) or Kearns Sayre (external ophthalmoplegia, retinopathy, myopathy and ataxia) syndromes. Interestingly, these syndromes show considerable overlap: children who survive the former can go on to develop the latter, perhaps reflecting a common pathway in their pathogenesis.

A variety of mutations in nuclear genes encoding mitochondrial proteins have been reported in the last few years that cause renal disease in children. Both tubulopathy[13] and nephrotic syndrome[14,15]

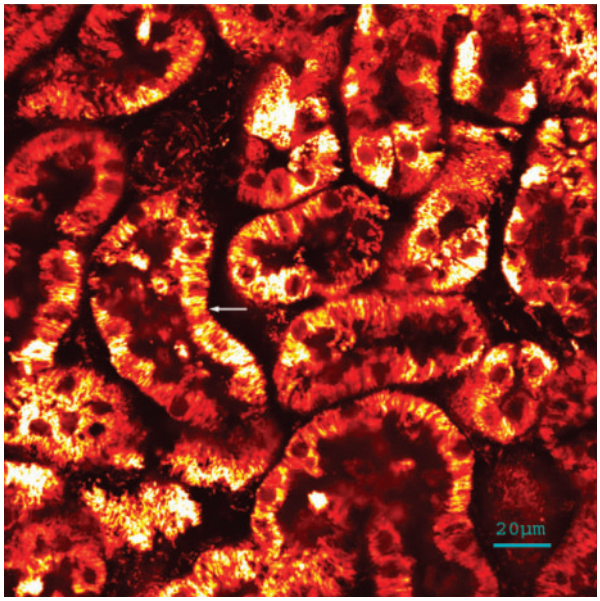

Figure 2. Multi-photon image of live rat kidney, stained with the mitochondrial dye TMRM, demonstrating the high density of mitochondria (arrowed) in proximal tubules, which lie in a striated baso-lateral pattern.

have been associated with co-enzyme (CoQ10 as ubiquinone) deficiency, and, more recently, mutations in two nuclear genes (*COQ2*[16] and *PDSS2*[17]) encoding components of the CoQ10 biosynthetic pathway have been described in patients with renal disease. Mutation of *BCS1L* can lead to impaired complex III assembly,[18] while mutation of *COX10* can lead to impaired complex IV activity;[19] both have been described in patients with proximal tubular disease. Recently, a mtDNA depletion syndrome causing tubular disease (due to a defect in the nuclear gene *RRM2B*[20]) has been described.

A screening study in children with MC showed that almost half had either FS or sub-clinical proximal tubulopathy,[11] suggesting that the burden of proximal tubular disease in these patients is significantly underestimated, whatever the underlying mutation. Measuring serum lactate has often been used as a screening test for MC, though in the presence of renal disease (FS) it may be normal owing to increased urinary loss of lactate, which is normally reabsorbed by the proximal tubule.[21] In children with multi-system MC the prognosis is often poor.

The reason for the high prevalence of proximal tubular disease in children is not known. The kidney is a highly aerobic organ, with a high density of mitochondria required for its transport activity and the reabsorption of >99% of the daily glomerular filtrate, most of it along the proximal tubule. The density of mitochondria varies along the nephron, and while it is high in the proximal tubule (Fig. 2), it is actually higher in more distal parts such as the thick ascending limb of the loop of Henle.[22] Perhaps the answer lies in the fact that the proximal tubule has a limited *anaerobic* glycolytic capacity and so it is dependent on *aerobic* metabolism for ATP generation.[23] In fact, it has been shown that a metabolic gradient exists in the tubules of the mammalian kidney, with aerobic metabolism predominant in the cortex, near the kidney surface, while anaerobic glycolysis predominates in the papilla; tubular cells in the outer medulla can use both forms of metabolism more or less equally.[24] However, there could be other mechanisms related to some of the specialized functions of the proximal tubule (e.g. receptor-mediated endocytosis) that render it more vulnerable to MC.

Adult renal disease and MC

The situation in adults with MC seems to be different from children. Although a range of renal pathologies has been reported, including tubulo-interstitial disease and multi-cystic disease, the most common finding has been focal segmental glomerulo-sclerosis (FSGS).[25–27] For reasons that are not yet clear, there appears to be a female preponderance in the literature, which has not been observed in children, and there is wide variation in age at presentation. FSGS has been described in children with MC,[28,29] but does not appear to be as frequent as tubular disease.

Involvement of other organs is common, but the severe multi-system disease seen in children is rare. Hearing loss seems to be particularly common and has led to some confusion with Alport's disease (which can be differentiated by the presence of haematuria and non-progressive hearing loss). Diabetes is also common and can be aggravated by steroids given to treat FSGS; renal histology suggests that diabetes is not responsible for the nephropathy.

In contrast to MC-related renal involvement in children, a significant number of adult patients seem to present with renal disease before a diagnosis of MC. Various clues can suggest the underlying diagnosis, including the presence of sub-clinical disease in other tissues, a positive family history (though not necessarily of renal disease) with maternal inheritance, a lack of response to conventional therapy, and the presence of abnormal looking mitochondria on electron microscopy (EM) of renal biopsy tissue; however, the morphology of mitochondria does not always correlate with their functional state, and phenotypic variation within

families can make maternal inheritance difficult to recognize.

In contrast to the heterogeneity of underlying mtDNA mutations in paediatric patients, only a single point mutation has been described in adult patients with renal disease: the A3243G mutation in the leucine^{UUR} tRNA gene. In addition to causing a variety of renal disorders, this mutation is associated with >80% of cases of MELAS syndrome (mitochondrial encephalo-myopathy lactic acidosis and stroke).[30] and MIDD (maternally inherited diabetes and deafness).[31] It has also been associated with cardiomyopathy[32] and external ophthalmoplegia[33] (Table 1). In renal patients, the prevalence of this mutation might be far higher than currently recognized: one study of diabetic patients on haemodialysis reported a prevalence of 5.9%[34] compared with an estimated prevalence of 0.24% in the general population.[35] Moreover, in a screening study of patients with MIDD, 28% were found to have renal involvement ranging from asymptomatic proteinuria to end stage renal disease.[36] The A3243G mutation is thought to account for ~0.06% of cases of diabetes mellitus in the UK.[37]

Mutations of mtDNA, including A3243G,[38] have also been described in patients with kidney tumours.[39,40] The extent and mechanism of any causal relationship is still unclear, but certain mtDNA haplotypes have been associated with renal cancers.[41] Reduced mtDNA content and RC activity have been described in renal cancers,[42] which may reflect a switch to more glycolytic metabolism by the tumour cells (the Warburg effect).

Table 1 Diseases associated with the A3243G mtDNA mutation

MELAS
Chronic kidney disease (FSGS)
MIDD
Cardiomyopathy
External ophthalmoplegia

Differences between children and adults

Clear differences between paediatric and adult patients with MC-related renal disease are beginning to emerge in the literature (Table 2). Children tend to present with a proximal tubulopathy at an early age, as part of an established and severe multi-organ disease due to mtDNA deletions (and/or nuclear mutations), and to have a poor prognosis. The mutations are generally sporadic and they are not usually passed on because of the early onset and severity of disease, and because survival into adulthood is rare (although, in general humans do not seem to pass on mtDNA deletions to their offspring for reasons that are still unclear).

To some extent the pattern of genetic mutations described causing MC and renal disease in children and adults reflects the general situation in MC; whereby nuclear mutations are more likely to present in childhood, whereas mtDNA mutations tend to present later in life (for a recent review of MC in childhood see[43]). Most nuclear mutations are expressed in all tissues and would be expected to cause severe multi-system disease. However, the clinical pattern of disease does not always follow the levels of expression of mutant genes in different tissues.[44] The reasons for this are still unclear; although a possible explanation is that expression levels may vary during embryonic development, resulting in particular patterns of disease in the newborn,[45] but this is unproven. Within the kidney, differential expression of affected proteins in different regions of the nephron (e.g. glomerulus vs. tubule) provides an attractive, though speculative, explanation for the spectrum of phenotypes seen in MC. In adults with point mtDNA mutations, heteroplasmy of mutant load among different tissues is an

Table 2 Comparison of the available published paediatric and adult literature on renal involvement in MC

	Children	Adults
Predominant renal involvement	Proximal tubule (FS)	Glomerulus (particularly FSGS)
Extra-renal features	Severe multi-system disease	Mild/sub-clinical (e.g. diabetes or deafness)
Underlying mutation	No clear pattern, often mtDNA deletion and/or nDNA mutation	Point mutation in mtDNA (A3243G)
Inheritance	Usually sporadic	Often maternal
Prognosis	Poor	Some initial success with transplantation

obvious explanation for the wide variation in phenotype, including severity and organ specificity.

Unlike children, adults tend to present with a milder phenotype and sometimes with only renal disease. Furthermore, renal pathology is more usually glomerular; in all cases the underlying defect is due to a single point mutation in mtDNA that can be maternally inherited. The reason why a particular pathology (FSGS) should occur in these cases is unknown, but the answer may lie in our increasing knowledge of podocyte biology. Any terminally differentiated cells with a heteroplasmic mtDNA pool will be vulnerable to MC, since cell division provides an opportunity to segregate mutant mtDNA unevenly between daughter cells, which can result in a clone of cells with a lower mutant load and a metabolic (and selective) advantage. Daughter cells with a higher mutant load may be recognized as damaged and deleted by apoptosis. Podocytes are terminally differentiated and unable to undergo regenerative proliferation in response to cell loss,[46] which might explain the relatively high prevalence of glomerular disease in adults with MC.

Why has only one mtDNA mutation been described so far in adult nephrology? This is surprising given the high rate of mtDNA mutation, and that >200 mtDNA mutations that have been described in other medical specialities (see MITOMAP: A Human Mitochondrial Genome Database. http://www.mitomap.org, 2006). It may reflect a lack of awareness of MC as a cause of renal disease; eventually many unexplained cases of chronic renal disease could be linked to other mtDNA mutations. In the 'mito-mouse', a mouse model of MC, another and unique mutation (a 4696 base-pair mtDNA deletion) caused FSGS,[47] but this mutation has not been reported in humans. Because it seems to be a common mtDNA mutation, A3243G is included in MC screening by most clinical genetics laboratories and this may bias towards a higher reported prevalence.

Pathogenesis of MC and renal disease

How might a mutation in mtDNA (such as A3243G) lead to very different disorders such as MELAS and FSGS? Some insight into the cellular consequences of mtDNA mutation has been gained from studies of transmitochondrial cybrids. These are immortalized human cells depleted of mtDNA by exposure to a nucleoside analogue such as 2′,3′dideoxycytidine (ddC)[48] that inhibits mtDNA replication. Donor mitochondria are then introduced by fusion with platelets or enucleated skin fibroblasts derived from patients with a known mtDNA mutation. This model has the advantage that the effects of mtDNA mutations on cell metabolism can be investigated without the confounding effects of unknown variations in nuclear genes encoding mitochondrial proteins.

In cybrids derived from patients with MELAS due to the A3243G mutation, significant reductions in mitochondrial translation products and impaired RC function occur.[49] The A3243G mutation has also been associated with a number of cellular biochemical defects in addition to reduced RC function, including increased oxidative stress,[50] impaired Ca^{2+} homeostasis,[51] reduced mitochondrial membrane potential,[52] abnormal post-transcriptional tRNA modification[53] and increased sensitivity to signals for apoptosis[54] (Fig. 3). Yet significant

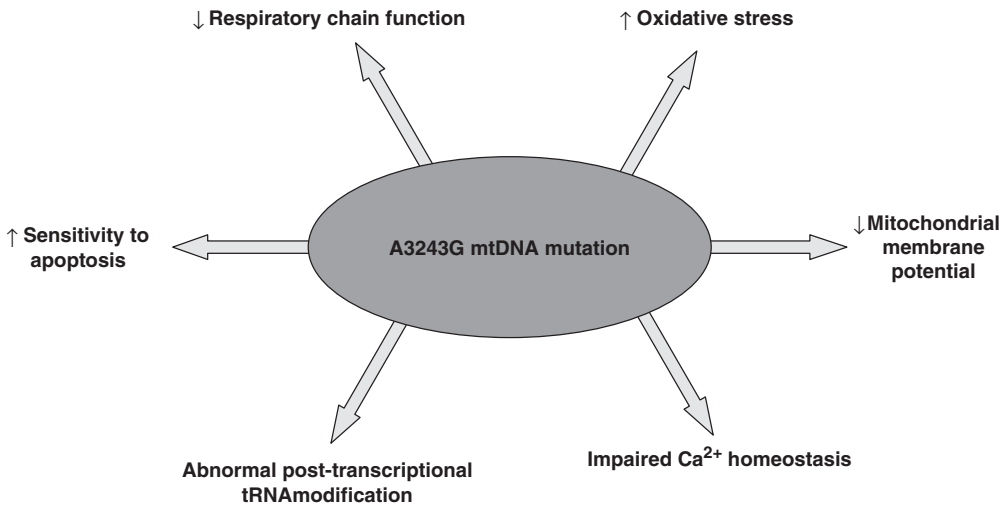

Figure 3. Intracellular changes associated with the A3243G mtDNA mutation.

protection was conferred by only a 6% level of wildtype mtDNA in the A3243G cybrids, indicating that a mutant load of ~95% is required to produce abnormalities in cultured cells, and perhaps also in disease. In patients with MELAS the mutant load varies widely among different cell types and individuals, and clinical disease can occur with much less than a 95% mutant load, which suggests that other factors are necessary *in vivo* for disease to manifest itself. This *in vitro* vs. *in vivo* difference may also reflect differences in vulnerability to mitochondrial dysfunction in immortalized cultured cells, as these tend to favour anaerobic glycolytic metabolism, which is a weakness of the cybrid system.

Recently the puromycin aminonucleoside nephrosis (PAN) rat model of FSGS has been used to explore the role of mitochondrial dysfunction in pathogenesis.[55] In this model, injections of puromycin aminonucleoside produced an early nephrotic phase (analogous to minimal change disease in humans), followed by the histological changes seen in FSGS. The authors found that the development of FSGS was associated with a reduction in whole kidney mtDNA and in mtDNA-encoded proteins in glomeruli. Abnormalities in mitochondrial morphology in podocytes were also noted on microscopy. How PAN causes these changes in mtDNA is unknown. In this model, nitric oxide (NO) levels rose and fell in phase with the changes in mtDNA, which might be important as NO can affect mitochondrial biogenesis through the generation of cGMP.[56] Though how these changes in mtDNA lead to mitochondrial dysfunction and the histological features of FSGS is not clear.

In the 'mito-mouse', although multi-organ disease does occur, the cause of death is renal failure at about 6 months of age.[47] Histology of the kidneys shows dilated proximal and distal tubules, as well as glomerular segmental sclerosis. RC function tests from affected kidneys indicate that COX activity is reduced to ~28% of normal. This model provides direct evidence for a link between a mutation of mtDNA, a reduction in RC function in the kidney and renal failure. Interestingly, the authors reported that the tubular disease was largely cortical, perhaps reflecting the gradient in anaerobic glycolytic capacity referred to earlier.

In spite of a major research effort, how MC can lead to disease at a cellular level is still unclear. One attractive theory is that mtDNA mutations may increase oxidative stress, which in turn causes oxidative damage to the RC, acting as a positive feedback mechanism that amplifies mitochondrial dysfunction.[57] Such a process could account for the slow progression of disease in many patients, despite the expression of the mutation from birth, but this is speculative.

Heterogeneity of MC phenotype

Clearly organs with a high rate of aerobic metabolism (e.g. the heart, skeletal muscle, brain and kidney) will be more susceptible to mitochondrial disease than cells or tissues with a higher anaerobic glycolytic capacity. Recent work has highlighted significant differences among these aerobic organs in mitochondrial density, their spatial distribution, morphology and oxidative phosphorylation capacity, which might also help to explain selective vulnerability and patterns of organ involvement with different types of MC.[58] Even within a tissue, variations exist among cell types in their relative dependence on aerobic metabolism. Studies using yeast (a cell model used extensively by mitochondrial biologists) have suggested that cells can adapt to their environment over time by losing mtDNA after prolonged exposure to anaerobic conditions. Furthermore, cybrid studies have supported the concept that the more anaerobic a host cell is, the more resistant it is to the effects of adding mutant mtDNA,[59] perhaps explaining why more distal parts of the renal tubule appear less vulnerable to MC. As explained above, cells that are terminally differentiated (like podocytes) will be prone to the effects of mtDNA mutations. Although these factors may explain why some tissues are more sensitive than others, they do not explain how a single point mutation can cause such a wide variety of phenotypes. What other explanations could there be?

The importance of the interaction between nuclear and mitochondrial genomes should not be underestimated. The mitochondrial DNA only encodes for a small proportion of mitochondrial proteins and replication; transcription and translation are dependent on nuclear encoded proteins that must be imported into mitochondria. It is thought that many of the nuclear-encoded mitochondrial genes currently residing in the nucleus were originally located in the mitochondrial genome, and have moved over time to the nucleus for greater protection and a reduced mutation rate. It may be that in patients with an identified pathogenic mtDNA mutation another, as yet unidentified, mutation in nDNA is necessary to co-exist or develop for disease to occur—a 'two hit' hypothesis (cf. polycystic kidney disease).

Another possible explanation for heterogeneity of phenotype is heteroplasmy. In muscle from MELAS patients mutant mtDNA content usually

exceeds 80%, though what governs the level of mtDNA mutation in a particular tissue is unclear. It is believed that when an oocyte containing mutant mtDNA is fertilized and divides, mtDNA is distributed randomly to each daughter cell. Cells with relatively higher mutant loads that go on to form particular tissues will increase the likelihood of organ-specific disease. Mutant load can also then vary among different cell types within the same organ, which may account for the occurrence of particular patterns of renal disease. For example, in a case of 'Bartter-like' syndrome in a child with MC (Kearns-Sayre syndrome), histo-chemical testing of the renal biopsy revealed that RC activity varied in different sections of the nephron: the collecting duct seemed to be relatively spared, allowing characteristic hypokalaemic alkalosis to develop.[60] Tissues containing cells that rapidly divide (e.g. lymphocytes) have the capacity to progressively reduce their mutant load by selecting for 'healthier' daughter cells after each cycle of division. The mutant load can be much higher in renal epithelial cells isolated from the urinary sediment than from blood in some patients with the A3243G mutation.[61] However, in the 'mito-mouse' levels of mutant mtDNA were similar in different organs, despite the heterogeneity in severity of organ disease.

Like many other diseases, the impact of the environment on the expression of MC also needs to be considered. Environmental toxins acting on a background of mutations in nDNA or mtDNA genes encoding mitochondrial proteins could affect the timing, severity and organ specificity of MC. For example, several toxins have been identified that may cause diseases such as renal FS by disturbing mitochondrial function: aminoglycosides,[62] ifosfamide[63] and heavy metals such as cadmium[64] are examples. The renal proximal tubule is particularly vulnerable to mitochondrial toxins, because it is a major site for the excretion of xenobiotics. Recent attention has focused on anti-retroviral therapy (ART). Nucleoside analogue reverse transcriptase inhibitors are thought to be toxic to mitochondria and have been reported to cause FS.[65] Furthermore, Tenofovir (a widely used *nucleotide analogue* reverse transcriptase inhibitor that originally was not thought to be toxic to mitochondria or the kidney) has now been associated with FS,[66,67] and may also potentiate the mitochondrial toxicity of certain other forms of ART.[68] As the treatment of HIV improves with ever more powerful ART, we may be entering a new phase in HIV-related nephropathy in which traditional glomerular HIVAN is being replaced by the tubulo-toxic effects of ART (and appropriate monitoring of these patients needs to be performed routinely). Understanding more about the effects of mitochondrial dysfunction in the nephron is clearly important and goes beyond simply trying to improve the management of patients with MC due to mtDNA mutations.

Although at first sight FSGS and the neurological features of MELAS seem to be very different, they may share an underlying patho-physiology. For example, evidence has emerged that the multi-focal infarcts located in the posterior cortex of the brain, which are typical of MELAS, may be due to mitochondrial disease in the vasculature supplying this region, rather than to a primary neuronal disorder;[69] although this remains controversial.[70] By analogy, renal vascular disease can produce features of FSGS on renal histology. Indeed, significant abnormalities in the renal vasculature are seen in renal biopsies from adult patients with FSGS and MC.[71]

Diagnosis, treatment and the future

From what we have learned in adult patients, it is likely that the prevalence of MC in the renal population has been underestimated, and that some cases of unexplained FSGS and other progressive renal diseases are the result of undiagnosed mtDNA mutations. Criteria for diagnosing MC have been suggested[72] and a simplified version of these is given in Table 3. However, these criteria have limitations and diagnosing MC with confidence can be very difficult, because there are no reliable or specific biomarkers, and because of the inherent variability and unspecific nature of presentation of MC. For example, serum lactate may be normal in renal disease due to lactate loss in the urine, and RC function tests can vary widely between individuals, and also tend to decline with age.[73] Non-renal manifestations of MC may occur before renal complications become apparent. In children, diagnostic criteria may need to be modified to take account of differences in clinical presentation between adults and children with MC.[74]

Functional MRI imaging of the CNS can be useful in some cases of MC.[75] For example, MR spectroscopy can detect excess metabolites in the brain such as lactate, which may be increased due to MC. Some cases of MC have been detected initially by characteristic brain CT/MRI findings (that help to distinguish them from other CNS diagnoses), and have subsequently been confirmed by mtDNA screening.[76,77] Functional MRI imaging of the kidney is currently less advanced than that of the CNS, and is still at an experimental stage; but it may offer hope for the future as a non-invasive tool to

Table 3 A summary of criteria commonly used to help identify patients with a MC

Criteria	Features	Comments
Clinical	Unexplained multi-system disease affecting predominantly 'aerobic organs'. (e.g. encephalo-myopathy, myocardial hypertrophy, renal FS).	Poor correlation between genotype and phenotype with high phenotypic variability. Disease may be inherited maternally or autosomally, or it may be due to a new mutation. Patients can have single organ disease (with or without sub-clinical involvement of other organs).
Biochemistry	Elevated serum lactate, pyruvate, creatine kinase. CSF lactate. Urinary amino acids.	Tests not specific of MC. Lactate may only increase on exercise, and can be normal in patients with a proximal tubulopathy.
Organ histology (usually muscle)	Presence of 'ragged red fibres'. Distorted mitochondrial architecture on EM.	Due to heteroplasmy of mtDNA mutant load, muscle may be normal, therefore try to biopsy organ(s) affected by disease clinically.
Immuno-histology	Can determine levels of sub-units of RC	Can help to differentiate between abnormalities in levels of nDNA vs. mtDNA encoded subunits.
RC function (Histo-chemical or spectrophotometric)	Activity of complexes I and/or IV typically reduced with mtDNA mutations, complex II sub-units all nDNA encoded.	High variability in RC function between individuals and different assays; tends to decline with age. Can only test for complexes II and IV with histo-chemistry.
Screening for mtDNA mutations	PCR for point mutations and Southern blotting for deletions.	Need to determine patho-genicity of mutations detected. Will not identify mutations in nDNA.

diagnose metabolic renal disorders such as MC. As myopathy is frequently present in MC, non-invasive screening tests to measure oxidative metabolism of muscle (such as the aerobic forearm test[78]) are currently being evaluated as a clinical aid to diagnosing MC.

Screening for mtDNA mutations can be performed by PCR and large mtDNA deletions can be detected by Southern blotting. The efficacy of mtDNA screening is limited to the number of mutations looked for. Although it is possible to completely sequence the mtDNA, this will not detect cases of MC due to mutations in nuclear genes encoding mitochondrial proteins. The choice of tissue is also important: blood and muscle are commonly used, as they are readily accessible and are often appropriate for patients with multi-system disease. However, due to the phenomenon of heteroplasmy discussed earlier, mutant load may vary in different tissues. Hence, a patient with low mutant load in rapidly dividing lymphocytes may have a higher mutant load in more differentiated cells of the kidney. RC function tests and mtDNA screening can be performed on tissue from a renal biopsy. A less invasive and alternative method for mtDNA screening involves using mtDNA from renal tubular cells collected from urine. It has been shown previously that in patients with the A3243G mtDNA mutation, a correlation exists between mutant load in muscle and urinary epithelial cells.[79]

Given the normally high rate of mtDNA mutation, it has to be demonstrated that any mtDNA mutation detected is truly pathogenic and responsible for disease. This can be ascertained by showing that a mutation in mtDNA exists at significant levels (usually, but not always, heteroplasmic), shows linkage with the phenotype, is not present in normal controls, and leads to an alteration in an evolutionary conserved rRNA, tRNA or amino acid residue. Further evidence can be provided by cybrid experiments in which transfer of a RC defect with a mtDNA mutation into a host cell supports a pathogenic effect. The importance of demonstrating a *functional* consequence of a novel mtDNA mutation was highlighted by a recent paper that questioned the pathogenecity of two previously described mtDNA deletions.[80]

A possible future approach is to create cybrids using mtDNA derived from patients with renal disease and host immortalized human renal cell lines, and to screen for functional biochemical defects. A technique like this could be used to detect evidence of mitochondrial dysfunction in cases of unexplained renal disease. Furthermore,

by using mtDNA from patients with known MC, the effects of mitochondrial dysfunction on the normal functions of renal cells (such as solute transport) could also be investigated. A similar approach has been tried in Alzheimer's disease,[81] but it is limited by the metabolic changes that can occur in cultured cells. Producing animal models with mtDNA mutations associated clinically with renal disease will clearly be of value.

At present there is no evidence-based treatment for MC, partly owing to the difficulty in conducting large scale trials in such a heterogeneous condition. A range of different therapies has been tried with some anecdotal reports of improvement (for review see[82]). Approaches used include ROS scavengers (e.g. vitamin E, glutathione), cofactors to enhance metabolism and ATP synthesis (e.g. vitamin B complex and C, L-carnitine, folic acid), and electron acceptors (e.g. vitamin C and E, succinate). Perhaps a reason why anti-oxidants and co-enzyme supplements have proved disappointing is because of difficulties in targeting them to the mitochondria. Regular exercise has been advocated in patients with MC and myopathy, and avoidance of drugs potentially toxic to mitochondria (e.g. metformin) is clearly important. Removal of excess metabolites that accumulate in MC could be beneficial; however, a trial of dichloroacetate to lower serum lactate in patients with the A3243G mutation had to be stopped because of peripheral nerve toxicity.[83] CoQ10 has been widely used as a therapy for MC. As a component of the normal RC it can act as both an acceptor of electrons and a ROS scavenger. In cases of MC resulting from CoQ10 deficiency, supplements can lead to a significant improvement in neuromuscular symptoms; however, they do not appear to affect the nephropathy that can occur in some of these patients. Perhaps this is related to the timing of any treatment, which might have to be given before renal damage is irreversible.[14,15]

Some novel experimental treatments are currently being considered, focusing on functions of mitochondria other than ATP production. Potential therapeutic targets include the mitochondrial permeability transition pore and the Bcl-2 family of proteins, which are involved in regulating apoptosis; while uncoupling proteins in the RC could be manipulated to reduce ROS production (for a review of these strategies see[84]).

Gene therapy to introduce wild-type mtDNA into a population of affected cells may be possible in the future. However, this approach is fraught with technical difficulties, not least an effective way of introducing mtDNA into mitochondria; though it is known that mitochondria can undergo frequent fusion and fission within a cell, leading to the exchange of genetic material.[85] Therefore, simply introducing wild-type mitochondria into an affected cell could, over time, lead to a reduction in mutant load, as the mtDNA is repeatedly replicated; alternatively, replication of the mutant mtDNA could be selectively targeted and inhibited.[86] Another possibility is to transfect wild-type mitochondrial genes into nDNA with post-translational targeting of the gene product to the mitochondria.[87]

Although all these various approaches are still experimental, they may provide a means of reducing the chances of transmitting mtDNA mutations from mothers to their offspring. Meanwhile, renal transplantation has been carried out in adults with MC with some success;[25] however, complications of immuno-suppressive therapy such as diabetes seem to occur at an earlier stage post-transplantation.

Conclusion

Much remains to be learned about the important links between mitochondrial dysfunction and disease; as our knowledge of MC grows, it is likely that the recognized burden of renal disease associated with MC will also increase. A greater understanding of mitochondrial function in the kidney will help us to devise new therapeutic strategies for various metabolic renal diseases, and especially those related to drug toxicity.

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