Diagnosis and Management of Mitochondrial Disorders

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Editors
Michelangelo Mancuso
Department of Clinical and
Experimental Medicine
Neurological Institute
University of Pisa
Pisa
Italy

Thomas Klopstock
Department of Neurology
Friedrich-Baur-Institute
Ludwig-Maximilians-University
of Munich
Munich
Germany

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Preface

Mitochondrial Medicine: 30 Years Old, Much to Learn

The initial spark of the "mitochondrial revolution" in medicine was the description, in 1988, of the first pathogenic mutations in mitochondrial DNA (mtDNA). Anita Harding and her team identified large-scale single deletions of mtDNA in patients with mitochondrial myopathies [1]. Soon thereafter, Doug Wallace and his team described a point mutation in the gene encoding subunit 4 of complex I in a family with Leber's hereditary optic neuropathy [2].

With the publication of this book in early 2019, we celebrate the 30th anniversary of these groundbreaking discoveries. The last 30 years have been the golden age of mitochondrial medicine, with hundreds of genes responsible for multiple genetic mitochondrial disorders being identified.

Mitochondrial diseases are now recognized as one of the most common genetic conditions worldwide, and the phenotypic expression involves all the disciplines of medicine.

We hope that we have been able to convey, with this book, the excitement that has accompanied—as it still does—the extraordinarily rapid development of mitochondrial medicine. The therapeutic era has just begun, and we are confident to see similarly exciting progress in the next few years.

It has been a great experience to serve as editors for this special book. We would like to express our special gratitude to all contributing authors for their timely and superb efforts in composing this monography.

Finally, this book is dedicated to our great mentor, Professor Salvatore "Billi" DiMauro. The enormous and still ongoing progress in our understanding of mitochondrial medicine is only possible by an intense collaboration of a team of international *mitochondriologists*, many of whom have been trained in the College of Physicians and Surgeons, Columbia University Medical Center, NY, under the guidance of Billi.

Enjoy the reading!

Pisa, Italy — Michelangelo Mancuso
Munich, Germany — Thomas Klopstock

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Mitochondrial Medicine: A Historical Point of View

Yi Shiau Ng, Salvatore DiMauro, and Doug M. Turnbull

Introduction

Mitochondria are essential double-membrane, dynamic organelles found in all nucleated cells, and they are referred as the powerhouse in cells because of their vital role in generating ATP via the oxidative phosphorylation (OXPHOS). The OXPHOS machinery is located at the inner mitochondrial membrane and comprises five enzymatic complexes, which are mitochondrial respiratory chain (complexes I to IV) and ATP synthase (complex V). The mechanism by which the passage of electrons down the respiratory chain generates ATP was described by Peter Mitchell [1], who was awarded the Nobel Prize for Chemistry in 1978. Mitochondria are also important players in multiple other cellular activities such as intrinsic apoptosis, redox, calcium handling and urea cycle.

One of the most fascinating biological features of mitochondria is that they contain extranuclear DNA materials, mitochondrial DNAs (mtDNA), which are tiny, double-stranded DNA molecules that exist in multiple copies per cell and only encode 37 genes. However, the replication and maintenance of mtDNA and almost all building blocks of mitochondria are controlled by the nuclear genome. The cross talk between the mitochondrial DNA and nucleus means that any genetic defects in either mtDNA or nuclear genome could perturb the mitochondrial functions especially the OXPHOS, consequently leading to the development of disease.

The clinical features of mitochondrial disease are very variable with high-energy demand tissues and organs such as the brain, skeletal muscle, heart, liver and optic nerves, which are particularly susceptible to the mitochondrial dysfunction. However, mitochondrial disease can affect practically any organ making the diagnosis and management challenging. Mitochondrial diseases are one of the most common groups of inherited neurogenetic disorders with a minimal prevalence of 1 in 4300 [2], comparably, if not, higher than other common neurogenetic disorders such as Charcot-Marie-Tooth neuropathy and myotonic dystrophy [3].

In this chapter, we begin with an overview of the pathological description of various mitochondrial syndromes, the biochemical classification of mitochondrial defects, followed by the era of identification of primary mtDNA mutations and discoveries of multiple nuclear genes implicated in mitochondrial disease. We also highlight the emergence of reproductive options especially mitochondrial donation in primary mtDNA disease and advancement in potential treatments (Fig. 1).

Y. S. Ng · D. M. Turnbull (✉)
Wellcome Centre for Mitochondrial Research, Institute of Neuroscience, Newcastle University, Framlington Place, Newcastle Upon Tyne, UK
e-mail: doug.turnbull@ncl.ac.uk

S. DiMauro
Houston Merritt Clinical Research Center, Columbia University, New York, NY, USA

Department of Neurology, College of Physicians and Surgeons, New York, NY, USA

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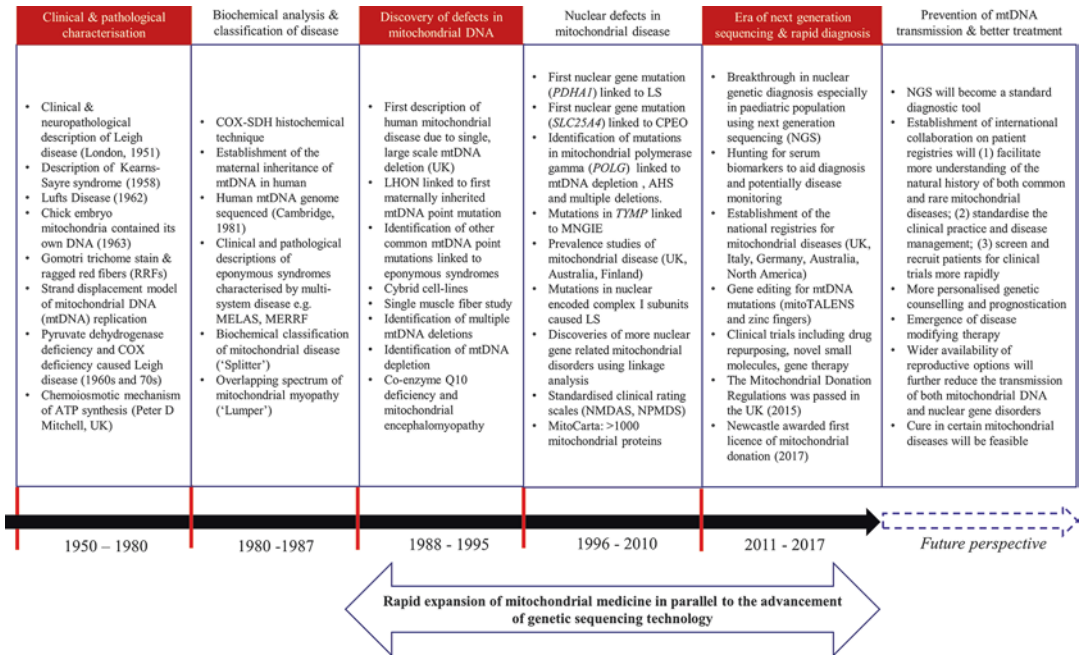


Fig. 1 Timeline summarises significant milestones and discoveries in mitochondrial disease. *AHS* Alpers-Huttenlocher syndrome, *ATP* adenosine triphosphate, *CPEO* chronic progressive external ophthalmoplegia, *COX* cytochrome c oxidase, *LHON* Leber hereditary optic neuropathy, *LS* Leigh syndrome, *MELAS* mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes, *MERRF* myoclonic epilepsy and ragged-red fibres, *mitoTALENS* mitochondrially targeted transcription activator-like effector nucleases, *MNGIE* mitochondrial neurogastrointestinal encephalopathy, *NGS* next-generation sequencing, *NMDAS* Newcastle Mitochondrial Disease Adult Scale, *NPMDS* Newcastle Paediatric Mitochondrial Disease Scale, *PDHA1* pyruvate dehydrogenase E1 alpha 1 subunit, *POLG* polymerase gamma, *SDH* succinate dehydrogenase, *SLC25A4* solute carrier family 25 member 4, *TYMP* thymidine phosphorylase

1950–1980

Leigh Syndrome

Leigh syndrome, also known as subacute necrotising encephalomyelopathy, is one of the most common presentations of mitochondrial disease among the paediatric patients with an estimated prevalence of 1 in 40,000 live births [4]. Doctor Denis Archibald Leigh (1912–1998), a talented British psychiatrist, published the first case report of clinical details and pathological findings of subacute necrotising encephalomyelopathy in London in 1951. He described a 7-month-old boy who had a normal birth and early development for 6 weeks, subsequently presented with a constellation of neurological signs and symptoms including developmental regression, poor feeding, optic atrophy and limb spasticity. A postmortem examination revealed bilateral symmetrical subacute necrotic lesions in thalami, brainstem and the posterior columns of the spinal cord with relatively sparing of the caudate and lentiform nuclei [5]. Leigh made an interesting observation that these pathological findings were very similar to patients with Wernicke's encephalopathy. The subsequent links of Leigh disease and inborn error of gluconeogenesis [6], cytochrome *c* oxidase deficiency (complex IV of respiratory chain) [7], pyruvate dehydrogenase complex deficiency [8] in the 1960s and 1970s implicated that Leigh syndrome did not result from a single molecular defect [7]. Indeed, mutations in more than 75 genes have been linked to Leigh syndrome to date [9].

Chronic Progressive External Ophthalmoplegia and Kearns-Sayre Syndrome

Chronic progressive external ophthalmoplegia (CPEO), characterised by eyelid ptosis and restricted eye movement, is now recognised as a common manifestation of mitochondrial disease (Fig. 2a) [10]. The German ophthalmologist,

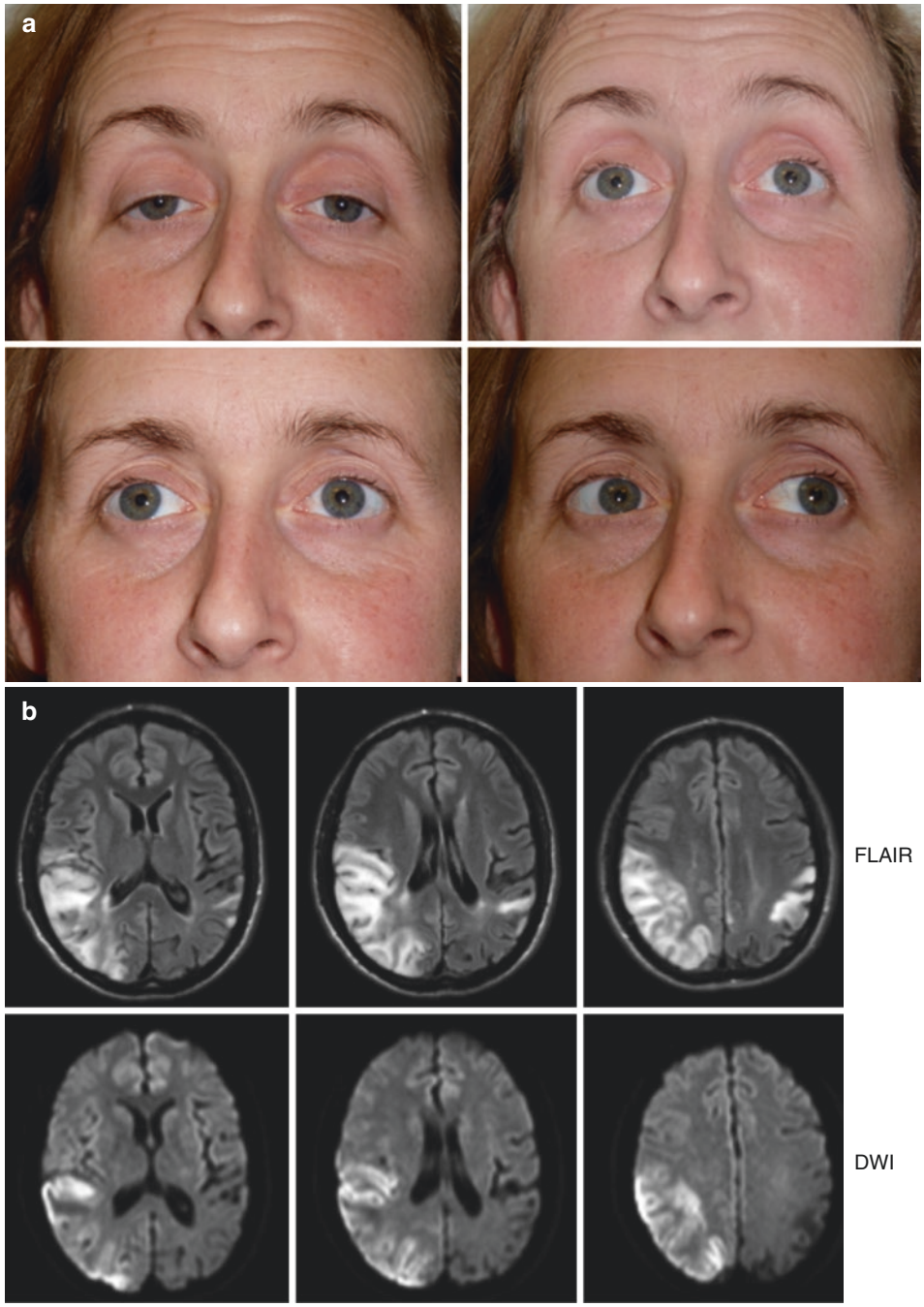
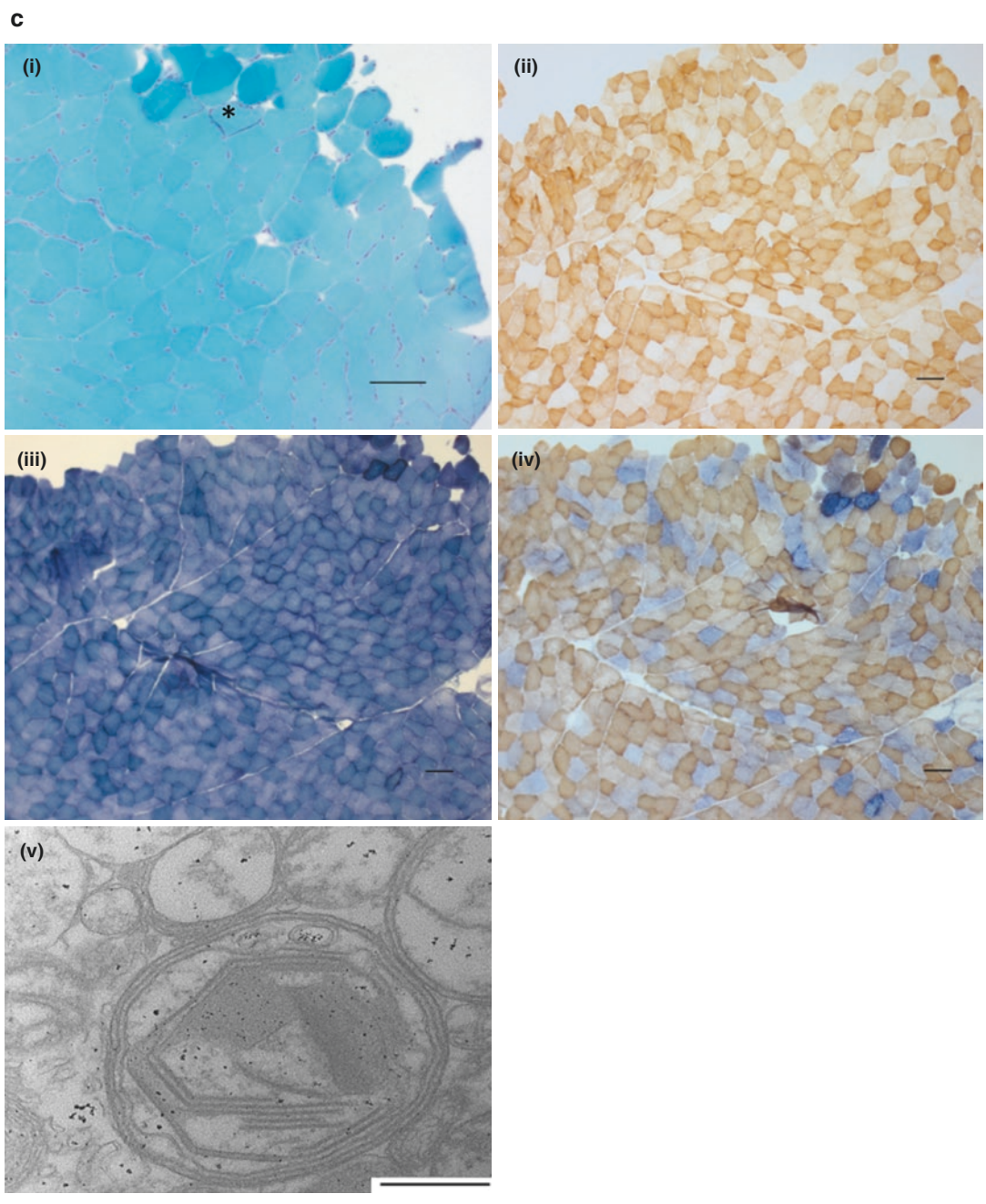


Fig. 2 (**a**) Signs of chronic progressive external ophthalmoplegia. This patient has bilateral ptosis, overactivity of frontalis, very limited upgaze, restricted abduction and adduction. (**b**) MRI head of a patient with MELAS syndrome. FLAIR sequence shows asymmetrical, bilateral stroke-like lesions with restricted diffusion involving the right temporal, parietal and occipital lobes. (**c**) Muscle biopsy. (1) A ragged-red fibre is highlighted with the modified Gomori Trichrome stain (asterisk, *); (2) COX-deficient muscle fibres exhibit pale brown colour; (3) increased SDH activities in COX-deficient fibres (darker blue); (4) sequential COX/SDH histochemistry clearly highlights the COX-deficient fibres (blue); (5) electron microscopy shows a highly abnormal mitochondrial ultrastructure. (**d**) Human mitochondrial DNA. Common point mutations including m.3243A>G, m.8344A>G, m.8993T>C/G, m.11778G>A and m.13513G>A and single, large-scale mtDNA deletion (4977 base pairs) are highlighted. (**e**) The prevalence of mitochondrial disease in an adult population of North East England. Over 75% of adult patients with mitochondrial disease are caused by a primary mtDNA defect [2]



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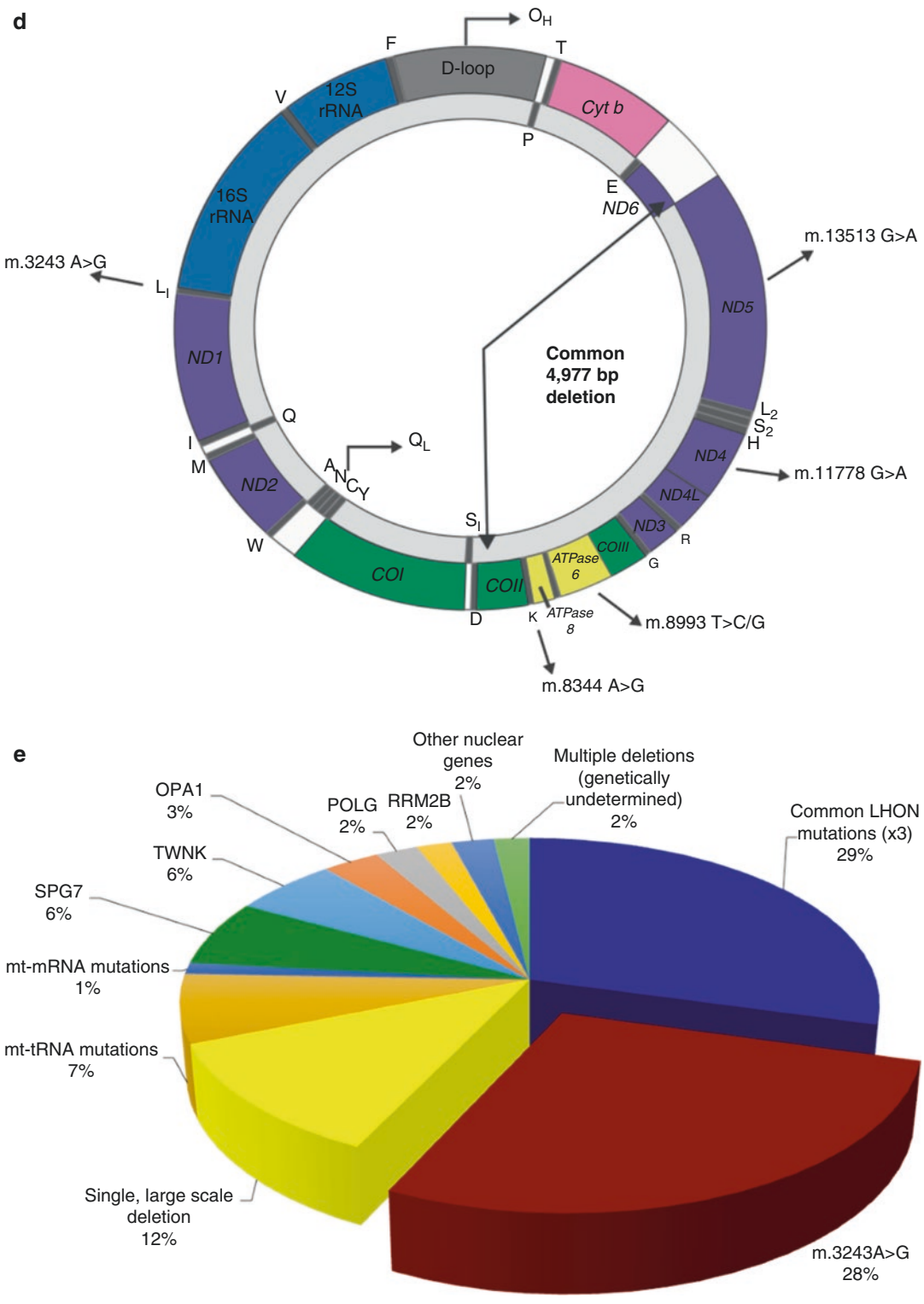

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Albrecht von Graefe, described the first case in 1868, and subsequently Sir Jonathan Hutchinson reported similar cases in the English literature around 10 years later. The underlying aetiology of CPEO had been widely but incorrectly accepted as a central brainstem disorder until histopathological and electromyographic evidence of myopathy in ocular muscles of affected individuals emerged in the early 1950s. In 1958, Kearns and Sayre from the Mayo Clinic reported two cases with triad of retinitis pigmentosa, CPEO and complete heart block, and they asserted that such association represented a true clinical syndrome rather than a coincidental finding [11]; Kearns reported nine more cases and outlined the spectrum of clinical features a few years later. Moreover, Kearns also observed the lack of family history in patients affected by this syndrome.

Luft Disease

The description of Luft disease in 1962 is often regarded as the beginning of the mitochondrial medicine [12]. The patient was a Swedish woman in her 30s presented with excessive perspiration, generalised muscle weakness and elevated metabolic rate with normal thyroid function. Muscle biopsy showed excessive accumulation of mitochondria, many of which had gigantic size. Further biochemical analysis and electron microscopy (EM) studies of mitochondria isolated from skeletal muscle directly linked the pathogenesis of disease to a defect involving in the coupling of oxidative phosphorylation [13].

A second case of Luft disease—with identical clinical, muscle pathology and biochemistry features—was reported [14], but the molecular genetic defect in this unique mitochondrial myopathy remains a puzzle.

Biochemical Classification of Mitochondrial Disease

The application of EM on studying muscle biopsies led to the discoveries that structurally abnormal mitochondria were identified in myopathies after the first description of Luft disease [15]. The availability of biochemical assays led to better characterisation of myopathies caused by various metabolic defects such as carnitine deficiency [16], carnitine palmitoyltransferase (CPT) deficiency [17], pyruvate dehydrogenase deficiency [18] and cytochrome *c* oxidase deficiency (complex IV) [7, 19, 20] in the 1970s and in the early 1980s. DiMauro and colleagues proposed to broadly classify mitochondrial disease into five major groups based on different steps of metabolic pathways in mitochondria [21]. Such classification encompassed a wide range of inborn metabolic disorders, which included pyruvate dehydrogenase deficiency, glycogen storage disorders, fatty acid oxidation defects and various mitochondrial respiratory chain deficiencies [21, 22].

1980–1987

The Mapping of Human Mitochondrial DNA

The presence of extranuclear DNA in mitochondria (i.e. mitochondrial DNA) in chick embryos was first reported by Nass and Nass in 1963. Maternal inheritance of mitochondrial DNA was identified in yeast and amphibians in the late 1960s and in mammals in 1974 [23]. Such inheritance pattern was confirmed in human in 1980 [24].

Sanger and colleagues who were based in Cambridge, UK published the complete sequence of human mitochondrial DNA, which has 16,569 base pairs, in 1981 [25]. They identified 22 tRNAs, 2 rRNAs, cytochrome *b*, 3 genes encoded for cytochrome *c* oxidase (CO I-III), ATPase 6 and 8 and 7 unidentified reading frames (URFs). They revealed that these genes were organised in a very compact fashion, and the noncoding region was located in the D-loop. The seven unidentified reading frames were subsequently identified to be subunits of complex I [26, 27]. It is highly remarkable that reanalysis of the Cambridge reference sequence only identified error frequency of 0.07% nearly 20 years later [28].

1989–2012

Mitochondrial Encephalomyopathies with CoQ_{10} Deficiency

In 1989, Ogasawara and Engel discovered two sisters with lipid storage myopathy, cerebellar ataxia, seizures and recurrent myoglobinuria and profound deficiency of CoQ_{10} in muscle mitochondria [29]. In the following years, many patients were reported with muscle CoQ_{10} deficiency and variable involvement of skeletal muscle, CNS, peripheral neuropathy, nephropathy and inconsistently responsive to CoQ_{10} supplementation.

It was suggested that various aetiologies of CoQ_{10} deficiencies should be attributed to genetic defects in the long series of enzymes involved in CoQ_{10} biosynthesis: in 2006 and 2007, the first molecular defects were identified in the genes (*PDSS1*, *PDSS2* and C*OQ2*) encoding the initial enzymes and causing severe infantile encephalomyopathies [30–32]. In the following years, mutations in *COQ8* explained the cause of adult-onset cerebellar ataxia, seizures, dystonia and spasticity [33–35], and several more genes have been associated with various forms of encephalomyopathies or nephropathies.

Secondary causes of CoQ_{10} deficiency have opened a new vista on ataxia, oculomotor apraxia (AOA1) due to mutations in *aprataxin (APTX)* [36] or on lipid storage myopathy due to mutations in electron-transferring flavoprotein dehydrogenase [37].

Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like Episodes (MELAS)

The acronym MELAS was first coined in 1984 [38], and it has become one of the most well-characterised syndromes in mitochondrial disease. Although the original case was presented at a paediatric neurology meeting in 1976, the full description of the original case of MELAS only became available 15 years later [39]. The diagnostic criteria of MELAS were proposed based on the literature review of 69 cases [39]: (1) stroke-like episode occurred before the age 40 years; (2) encephalopathy characterised by seizures, dementia or both; (3) lactic acidosis, ragged-red fibres or both; (4) normal early development; (5) recurrent headache; and (6) recurrent vomiting. Stroke-like lesions often do not confine to the vascular territories, with the predilection of occipital, parietal and temporal lobes involvement (Fig. 2b). These unique characteristics have been consistently observed in both the imaging [40–42] and neuropathological [43–46] studies. The precise pathogenesis remains debatable [47], and the leading hypotheses are angiopathy and endothelial dysfunction [48, 49], neuronal hyperexcitability [50] and inherent OXPHOS dysfunction caused combined neuronal and vascular dysfunction [44].

1988–1995

Mutations in the Mitochondrial DNA

The clear demonstration of mutations in mitochondrial DNA that was responsible for human disease only occurred in 1988: sporadic form of CPEO caused by the single, large-scale mtDNA deletion [51, 52]. In the same year, Wallace and colleagues demonstrated that Leber hereditary optic neuropathy (LHON) was caused by the maternally inherited homoplasmic mtDNA point mutation (m.11778G>A) in multiple unrelated family pedigrees for the first time [53]. Following these breakthrough discoveries, many clinical syndromes were linked to specific mtDNA mutations, such as m.8344A>G with myoclonic epilepsy and ragged-red fibres (MERRF) [54], m.3243A>G with MELAS [55], single, large-scale mtDNA deletion associated with Kearns-Sayre syndrome (KSS) [56] and Pearson syndrome [57] and other common point mutations causing LHON (Fig. 2d) [58].

Hammans and coworkers from Queen Square, London, demonstrated mtDNA mutations (m.3243A>G and m.8344A>G) were detectable in both blood and muscle and proposed to employ the molecular analysis of blood sample as a rapid screening and diagnostic tool for suspected cases in the early 1990s [59]. However, the mutant het-

eroplasmy level of several common point mutations such as m.3243A>G [60] and m.13513A>G has subsequently been shown to decline with time in blood, highlighting the caveat of a false-negative result by screening mtDNA mutations using blood sample alone. Other noninvasive tissues such as urine, buccal mucosa and hair follicles have since been proposed as alternative diagnostic samples to skeletal muscle and blood. Nevertheless, muscle biopsy (Fig. 2c) is important in the investigation of primary mtDNA disease, especially among individuals without apparent maternal family because single, large-scale deletion and sporadic point mutations in mtDNA can only be reliably detected in postmitotic tissues [61].

The advent of transmitochondrial cybrid cell study [62] and single muscle fibre analysis of mtDNA variant [63] have become the gold standard of ascertaining the pathogenicity of any novel mtDNA variants, given multiple polymorphisms are present in the mtDNA. The expansion of clinical spectrum associated with a given mitochondrial DNA mutation, for example, MELAS [55], MIDD [64] and CPEO [65] in patients with the m.3243A>G mutation, and genetic heterogeneity for the same clinical syndrome have been increasingly observed over time.

The mtDNA Bottleneck and Challenge in Genetic Counselling

The variations in mutant heteroplasmy level between generations are frequently observed, and the degree of variations differs between the mutations. Such observation leads to the theory of the mitochondrial genetic bottleneck, which hypothesises that only a small proportion of the maternal mitochondrial genome is transmitted to the offspring [66]. It is increasingly evident that size of bottleneck varies between the mtDNA mutations, and a recent simulation study based upon a compilation of heteroplasmy levels from family pedigrees published in the literature and unpublished data clearly demonstrated that the rate of random genetic drift varies between mutations [67]. Tighter genetic bottleneck, such as in the case of m.8993T>G/C mutation in *MTATP6*, indicates a more rapid segregation of mtDNA heteroplasmy between generations, which explains a common scenario encountered in the clinical practice that a severely affected child with very high/near homoplasmic mutant heteroplasmy born to an asymptomatic mother who carries very low mutant load [67].

1996–2010

Maintenance Defects of Mitochondrial DNA

The maintenance and replication of mtDNA are entirely dependent on machineries encoded by the nuclear genome. Defects in these machineries result in a myriad of human disease characterised by multiple deletions and/or depletion of the mtDNA copy number in postmitotic tissues [68]. Shortly after the report of sporadic, single large-scale mtDNA deletion in 1988, there was an important observation of multiple deletions in muscle biopsies and late-onset, autosomal dominant CPEO identified in several Italian families [69, 70]. The first nuclear gene reported to cause dominant, late-onset CPEO is *SLC25A4*, which encodes for the ADP/ATP translocase 1, in 2001 [71]. On the following year, a major discovery made by Van Goethem and coworkers in Belgium was the identification of dominant and recessive mutations in *POLG*, encoding for mitochondrial polymerase gamma, caused multiple deletions in mtDNA and CPEO [72]. Mutations in *POLG* have also been associated with wider phenotypic spectrum including devastating infantile-onset Alpers syndrome, ataxia neuropathy spectrum and myoclonic epilepsy, myopathy and sensory ataxia [73], Parkinsonism and premature ovarian failure [74]. The link of POLG deficiency and mitochondrial disease is significant, as highlighted by further genetic studies that the p. Trp748Ser pathogenic variant is the founder mutation of ancient European origin with the population carrier rate of 0.8% in Finland [75] whilst the p.Ala467Thr variant can be identified in 0.69% of the British population [76]. To date, at least 14 nuclear genes have been associated

with multiple deletions and CPEO phenotype of mitochondrial disease [77].

The reduction of the mtDNA copy number, also known as mtDNA depletion, was recognised as a distinctive cause of severe, infantile-onset mitochondrial disorder [78, 79] around the same time as the identification of multiple deletions in mtDNA. Broadly speaking, mitochondrial depletion syndrome is associated with four major clinical phenotypes: hepatocerebral syndrome, encephaalomyopathy, pure myopathy and neurogastrointestinal involvement [80]. The underlying molecular mechanisms include impairment in the mtDNA replication (e.g. *POLG*, *POLG2* and *TWNK*) and defects in the mitochondrial deoxynucleotide (dNTP) pool regulation (e.g. *TK2*, *DGUOK*, *RRM2B* and *TYMP*) [81]. The pathogenesis of mtDNA depletion remains elusive in some genes such as *MPV17* [82].

Clinical Rating Scales for Longitudinal Study

Whilst there are subtypes of mitochondrial disease present with isolated tissue or organ involvement such as LHON [83] and hypertrophic cardiomyopathy [84], multisystem involvement is evident in many patients when their disease progresses. However, longitudinal data detailing the disease trajectory has been generally lacking, hindering the effort of developing standardised guidelines for disease surveillance, genetic counselling and patient enrolment for clinical trials. Clinical rating scales for both adult [85] and paediatric [86] patients have been developed to address these unmet needs. The Newcastle Mitochondrial Disease Adult Scale (NMDAS) has been successfully applied on modelling disease progression of single, large-scale mtDNA deletion [87].

Establishment of the Prevalence of Mitochondrial Disease

The estimated minimal birth prevalence of mitochondrial disease is 1 in 5000 in the population, based on findings derived from two separate studies performed based on North East England and South Eastern Australia populations in the early 2000s [88–90]. Studies consistently show that in adults mtDNA mutations are more prevalent, whilst autosomal recessive nuclear defects are more common in children (Fig. 2e) [91]. A subsequent study that screened over 3000 neonatal cord blood samples from sequential live births in Northern England showed that the carrier rate of common pathogenic mtDNA mutations is 1 in 200 [92]. The discrepancy between the number of mutation carriers and clinically manifesting cases reflects that many people may harbour the mutant mtDNA heteroplasmy level below the expressing threshold and remain asymptomatic throughout their life; however, the maternal transmission of mtDNA mutations may continue inconspicuously in several generations until a proband is identified clinically.

2011–2017

Revolution of Genetic Diagnosis with the Next-Generation Sequencing

There are more than 280 nuclear genes that have been associated with mitochondrial disease to date [93, 94]. It is anticipated that more disease-causing genes will be discovered in the coming years because over 1100 proteins are localised to mitochondria, according to the inventory of mammalian mitochondrial proteins, MitoCarta 2.0 [95]. The nuclear-related mitochondrial disease can be classified based upon our understanding of the protein function, secondary defects in mtDNA and downstream biochemical defects in the OXPHOS [96, 97]. Isolated complex deficiencies are usually secondary to the defects in the structural subunits or assembly factors; in stark contrast, combined mitochondrial respiratory chain deficiencies are associated with multiple genes and pathways [98].

Next-generation sequencing (NGS), a new and high-throughput technique that allows sequencing of multiple candidate genes simultaneously, is leading to a more rapid diagnosis and increase the diagnostic yield [99]. The success of

whole exome sequencing (WES) in mitochondrial disease has been reported to range from 17% to 55%, depending on the patient selection criteria [100–102]. One of the greatest challenges with the NGS is to provide proof of pathogenicity for novel variants in the known genes and perhaps more so for the new genes that have not been previously linked to any disease. Segregation study of affected and unaffected family members would help to prioritise the analysis of variants of unknown significance (VUS). Detailed understanding of clinical phenotypes and identification of other affected individuals from different pedigrees are the pivotal step of validating the diagnosis [100]. Multicentre collaboration is often required to identify these patients because many of these VUS are rare. In the circumstance of private mutations for which segregation study cannot be performed, further in vitro studies such as Western blotting, mutant cell characterisation, rescue experiment and animal modelling are required [103].

Biopsies of affected tissues and biochemical measurement of these samples are invaluable when interpreting the WES findings, and they would continue to have a major role in the diagnostic workup in mitochondrial disease for the foreseeable future. However, it is also increasingly recognised that other genetics or 'acquired' neuromuscular diseases could mimic mitochondrial disease in terms of their clinical manifestations and muscle biopsy findings [104–107], again highlighting the complexity of investigating patients with evidence of 'mitochondrial dysfunction' in some cases.

Natural History and Cohort Studies

Improvement in the diagnostic strategies with the application of NGS has solved the diagnostic conundrum of many cases of mitochondrial disease. However, risk stratification and surveillance for complications, prediction of disease progression and prognostication remain extremely challenging in the clinical setting. The limitations in the longitudinal and natural history data have created significant barriers to developing medical management guidance, determining the timing of therapeutic trial and outcome measures, which are patient-centred and clinically relevant. Furthermore, more stringent patient selection would restrict the patient recruitment from a single source, and multicentre collaboration would be imperative to achieve sufficient sample size especially for randomised controlled trials (RCT) [108]. Leading mitochondrial research groups in the UK [109], Italy [110], Germany [111], the USA and Australia have established their respective national registry of mitochondrial diseases with the endeavour to elucidate the natural history of various genotypes better and prepare for patient enrolment to clinical trials since the late 2000s.

Treatment and Emerging Therapies for Mitochondrial Disease

The Cochrane review of published clinical trials concluded that there was no evidence-based treatment for mitochondrial disease in 2012 [112]. Although there remains no cure for mitochondrial disease, there are organ-specific supportive treatments [91] that could offer alleviation of symptoms (e.g. hearing aids and cochlear implant for sensorineural deafness, ptosis surgery), reduction of disease burden (e.g. pharmacological therapy for cardiomyopathy, insulin for diabetes mellitus, antiepileptic drugs for stroke-like episodes and/or seizures) and potentially life-saving treatment (e.g. solid organ transplant [113]). Targeted treatments are available for several forms of mitochondrial disorders such as allogenic haematopoietic stem cell transplant [114] and liver transplant [115] for mitochondrial neurogastrointestinal encephalopathy caused by *TYMP* mutations, supplementation of N-acetylcysteine and metronidazole for the ethylmalonic encephalopathy [91]. The dietary supplementation of vitamins and cofactors such as riboflavin, thiamine and ubiquinone has shown clinical benefits for specific groups of mitochondrial disorder [116]; however, these findings are unlikely to be validated in large-scale RCTs given the inherent small number of patients.

Idebenone, an antioxidant and inhibitor of lipid peroxidation, is the first orphan drug that was approved for the marketing authorisation by the European Medicines Agency (EMA) for patients affected by LHON in 2015, following the report of the largest, randomised controlled trial (n = 85) [117] and additional data derived from the expanded access programme and case record survey [118]. Advancements in the therapeutic research for LHON are prominent in recent years, especially the gene therapy using the recombinant adeno-associated virus. In vitro study [119] and early-phase clinical trials [120] have demonstrated the safety profile and observation of visual improvement, phase III, multicentre clinical trials are currently recruiting patients to confirm the therapeutic efficacy (ClinicalTrials.gov Identifier: NCT02652780, NCT03293524).

Molecular bypass therapy aiming to restore deoxyribonucleoside triphosphate (dNTP) pools [121, 122] is emerging as a novel treatment for *TK2*-related mitochondrial depletion syndrome characterised by severe myopathy. Other nuclear gene defects implicated in the nucleoside metabolism such as *RRM2B* may also benefit from the molecular bypass therapy in theory; however, neither animal nor clinical data is currently available to support its efficacy. On the other hand, several ongoing clinical trials are evaluating small molecules including novel compounds and repurposing drugs that aim to promote mitochondrial biogenesis, stabilise mitochondrial membrane or improve efficacy of scavenging reactive oxygen species [91, 98, 123]. Although small molecule therapy is generic and unlikely to be curative, it may be more cost-effective for the drug discovery and could potentially benefit more patients and have wider applications in other neurodegenerative disorders.

Zinc finger nucleases (ZFN) [124] and transcription activator-like effector nucleases (TALENS) [125] have been used experimentally to manipulate the ratio of mutant and wild-type mtDNA in cell lines and have shown an impressive reduction of mutant heteroplasmy level below the phenotypic expression threshold. Furthermore, the use of mitoTALEN has been attempted in the mouse germ line and provided proof of concept of its potential efficacy in preventing mtDNA transmission [126]. However, neither technique would be applicable to homoplasmic mtDNA mutations nor substantial reduction in the mtDNA copy number in cell lines with subsequent recovery raises a severe concern of safety in vivo.

Reproductive Options and Mitochondrial Donation

Nuclear gene-related mitochondrial disease follows the Mendelian inheritance rules, and the risk calculation of disease recurrence can be determined unequivocally. In contrast, the prediction of transmission risk is exceptionally challenging for heteroplasmic mtDNA mutations because of the random nature of mtDNA genetic bottleneck. Several reproductive options are currently available for heteroplasmic mtDNA mutations such as prenatal diagnosis and preimplantation genetic diagnosis (PGD). The success of PGD predominantly relies on selecting embryos created via in vitro fertilisation (IVF) to harbour mutation load below the threshold level expected for the individual mtDNA mutation [94]. However, these options are not appropriate for women who harbour very high mutation load or homoplasmic mutation, which have led to the innovative development of mitochondrial donation (aka mitochondrial replacement therapy).

Mitochondrial donation is an IVF-based technique that requires healthy donor oocyte and can be performed before fertilisation using metaphase II oocytes (maternal spindle transfer, MST) or after fertilisation using pronucleate stage zygotes (pronuclear transfer, PNT). Both methods result in an embryo that contains wild-type mtDNA predominantly from the donor, hence significantly reducing the risk of transmitting mutated mtDNA whilst retaining nuclear DNAs from the biological parents [94, 127]. PNT is the technique pioneered in Newcastle [128], and a recent preclinical study with the refined method has affirmed its safety profile with a note of caution that the prevention of mutated mtDNA transmission is not guaranteed [129]. In the UK,

mitochondrial donation is now a feasible reproductive option in the clinical setting after the extensive scientific and ethical scrutiny of the technique, but more crucially, the law change initiated by the active campaigning participated by patient groups, general public and the scientific community [130].

Nonhuman primate and more recent preclinical data [131] using MST method have provided some encouraging results of its safety and efficacy of preventing the transmission of mtDNA mutation. A healthy baby boy was born via the MST technique performed by the US-based medical team in Mexico in 2016; the mutant heteroplasmy levels were reported to range from 2.36% to 9.23% in different tissues [132]. Whilst this news generated a global interest on the first successful attempt of mitochondrial donation in human, this causes controversies in terms of ethical and legal considerations [133, 134].

Conclusions

The field of mitochondrial medicine has grown exponentially in the last few decades. Clinical description and pathological characterisation of individual syndromes have laid a strong foundation for the discovery of underlying genetic defects and uncovered the complexities of the dual genomic control of mtDNA, mtDNA replication and maintenance. Identification of the genetic mutations will no longer be an arduous undertaking for both patients and clinicians, with the advent of high-throughput next-generation sequencing technologies and bioinformatics. Our understanding of tissue specificity related to the underlying molecular genetic defect, phenotypic heterogeneity and epigenetics will hopefully be clarified further with better modelling systems and data derived from the omic technologies [135]. International, cross-disciplinary collaborations such as sharing of genomic data [136] and the establishment of global patient registry would facilitate the elucidation of the natural history of many mitochondrial disorders, standardisation of patient care, finding better prognostic biomarkers and perhaps, more importantly, expediting patient recruitment for the increasing number of therapeutic trials. Selection of robust outcome measures [137] and innovation of trial design will be crucial to maximising the success of translating bench findings into the clinical practice but to also reduce the burden on patients. The availability of various reproductive options including mitochondrial donation and potentially other mtDNA heteroplasmy-shifting techniques will lead to the reduction of the transmission of mtDNA mutations and eventually the prevalence of mtDNA disease.

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