MITOCHONDRIAL DNA MUTATIONS IN HUMAN DISEASE

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Abstract | The human mitochondrial genome is extremely small compared with the nuclear genome, and mitochondrial genetics presents unique clinical and experimental challenges. Despite the diminutive size of the mitochondrial genome, mitochondrial DNA (mtDNA) mutations are an important cause of inherited disease. Recent years have witnessed considerable progress in understanding basic mitochondrial genetics and the relationship between inherited mutations and disease phenotypes, and in identifying acquired mtDNA mutations in both ageing and cancer. However, many challenges remain, including the prevention and treatment of these diseases. This review explores the advances that have been made and the areas in which future progress is likely.

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Until recently, mitochondrial genetics and diseases that are due to defects in mitochondrial DNA (mtDNA) were often considered to be curiosities, outside mainstream genetics. These views were not helped by the different genetic rules by which mtDNA is inherited compared with nuclear DNA, the presence of several mtDNA copies in individual cells and a belief that mtDNA disease is rare. In addition, the clinical syndromes caused by mtDNA mutations have variable phenotypes and are often described by instantly forgettable eponyms or acronyms[1]. These views perhaps ignore the fact that the mitochondrial genome is central to the study of evolutionary genetics, has an important role in forensic medicine[2] and that the human, bovine and mouse mitochondrial genomes were the first mammalian genomes to be completely sequenced[3–5].

The increasing ease with which the mitochondrial genome can be analysed, and the availability of a consensus human sequence[6], have both helped to recognize mtDNA disorders as a frequent cause of genetic disease. It is difficult to estimate the true prevalence of mtDNA disease owing to its many clinical guises, presentations and the involvement of numerous causative mutations. Data on a single-point mutation (3243A>G) in Finland indicate that approximately 1 in 6,000 individuals are affected, whereas estimates from the British population intimate that about 1 in 3,500 people either have mtDNA disease or are at risk of developing it[7,8]. These estimates do not include the recent apparent association of mtDNA mutations and common clinical features (for example, hypertension)[9], which indicate that the incidence could be higher still. In addition, there is increasing evidence from animal models[10] and human studies[11] that acquired mtDNA mutations and mitochondrial dysfunction are involved in ageing and age-related diseases such as diabetes[12,13]. Although our present knowledge already indicates that mtDNA mutations are an important cause of disease, the true impact of these mutations on human health remains to be determined.

This review focuses on abnormalities of the mitochondrial genome in relation to human disease. Mitochondrial disease can also arise from nuclear gene disorders because most proteins involved in mitochondrial metabolism and all those involved in mtDNA maintenance are nuclear-encoded. Mutations in these nuclear genes can mimic the features seen in patients with mtDNA defects, and indeed some nuclear genetic disorders result in secondary abnormalities of the mitochondrial genome. Excellent reviews describe the defects that are due to mutations in nuclear genes that are involved in mitochondrial oxidative metabolism[1,14,15]. Here we review our current understanding of the role of mtDNA in human disease and highlight areas that are either controversial or merit further studies. We first review the basic features of mitochondrial genetics. Much progress has been made in this area

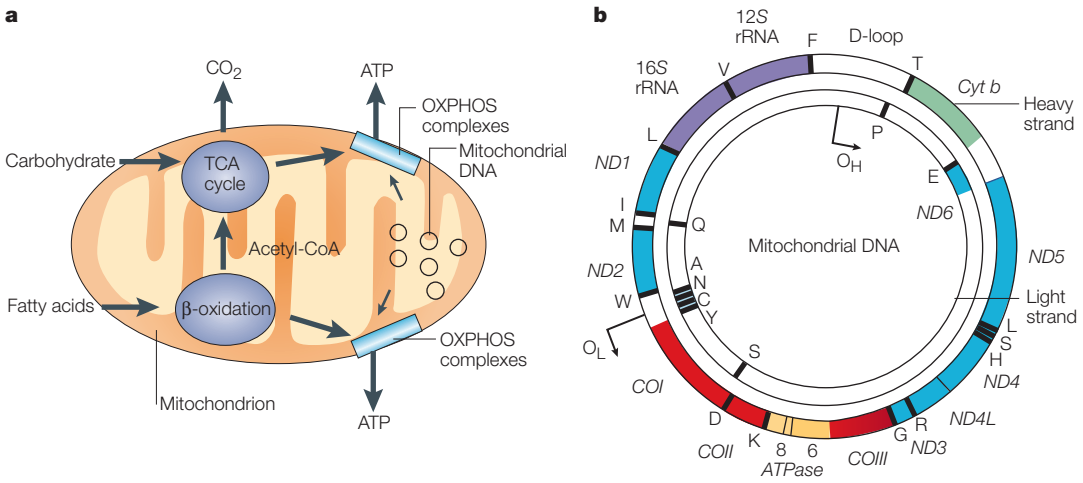

Figure 1 | **The role of the mitochondrial genome in energy generation. a** | This highlights the importance of the mitochondrial genome in contributing polypeptide subunits to the five enzyme complexes that comprise the oxidative phosphorylation (OXPHOS) system within the inner mitochondrial membrane — the site of ATP synthesis. The reoxidation of reducing equivalents (NADH (reduced nicotinamide adenine dinucleotide) and $FADH_2$ (reduced flavin adenine dinucleotide)) that are produced by the oxidation of carbohydrates (the tricarboxylic acid (TCA) cycle) and fatty acids (β-oxidation) is coupled to the generation of an electrochemical gradient across the inner mitochondrial membrane, which is harnessed by the ATP synthase to drive the formation of ATP. **b** | A map of the human mitochondrial genome. The genes that encode the subunits of complex I (*ND1–ND6* and *ND4L*) are shown in blue; cytochrome *c* oxidase (*COI–COIII*) is shown in red; cytochrome *b* of complex III is shown in green; and the subunits of the ATP synthase (*ATPase 6* and *8*) are shown in yellow. The two ribosomal RNAs (rRNAs; 12*S* and 16*S*, shown in purple) and 22 tRNAs, indicated by black lines and denoted by their single letter code, which are required for mitochondrial protein synthesis are also shown. The displacement loop (D-loop), or non-coding control region, contains sequences that are vital for the initiation of both mtDNA replication and transcription, including the proposed origin of heavy-strand replication (shown as O_H). The origin of light-strand replication is shown as O_L.

and a clear understanding is important to further our knowledge of the diagnosis, pathogenesis and treatment of mtDNA disease. We then turn to clinical syndromes that are due to mtDNA mutations, recognizing that mtDNA mutations might cause various clinical features, which often makes diagnosis a considerable challenge. Even when diagnosed, there is no curative treatment for patients who have mtDNA disease. Nonetheless, there are several experimental possibilities to either prevent transmission or to correct the genetic defect and these are discussed here. Although models of mtDNA disease exist, these are predominantly cell-based rather than transgenic mouse models. Some valuable models are available, but few mimic the characteristics of pathogenic mtDNA mutations. We conclude by considering the possible role of the mitochondrial genome in ageing, which, despite much speculation, has only recently been supported by firm evidence from an animal model.

Mitochondrial genetics: the basics

Mitochondria are found in all nucleated cells and are the principal generators of cellular ATP by oxidative phosphorylation (OXPHOS), incorporating the electron-transferring respiratory chain (complexes I–IV) and the ATP synthase (complex V). Mitochondria are the only location of extra-chromosomal DNA within the cell (except in plant chloroplasts), and they are under the dual genetic control of both nuclear DNA and the mitochondrial genome. The mitochondrial genome consists of a multicopy, circular dsDNA molecule (16.6 kb in humans), which encodes 13 essential polypeptides of the OXPHOS system and the necessary RNA machinery (2 rRNAs and 22 tRNAs) for their translation within the organelle (FIG. 1). The remaining protein subunits that make up the respiratory-chain complexes, together with those required for mtDNA maintenance, are nuclear-encoded, synthesized on cytoplasmic ribosomes, and are specifically targeted and sorted to their correct location within the organelle.

Mitochondrial genetics is different from Mendelian genetics in almost every aspect, from the uniparental inheritance of disease mutations, to the presence of many copies of the genome within a single cell and the basic mechanisms that underlie replication and control of transcription (TABLE 1). The differences between the two genetic systems that are in human cells are probably a relic of evolution[16], but lead to some fascinating biology that dictates the functional consequences of mtDNA mutations.

Replication, transcription and translation of mitochondrial DNA. Both replication and transcription are important in terms of understanding the development of mtDNA mutations and their biochemical consequences. There is now good evidence to indicate that the minimal mitochondrial replisome[17] consists of several proteins. These include: the heterodimeric mtDNA polymerase γ, which consists of a catalytic subunit with proof-reading ability (PolgA) and a processivity subunit (PolgB)[18], Twinkle, which has 5′–3′ DNA helicase activity[19], and a mitochondrial single-stranded binding protein. Together with mitochondrial transcription factor A (TFAM), these proteins associate with mtDNA to form structures

that are termed NUCLEOIDS, which are believed to be the units of mtDNA transmission and inheritance.

The mode of mtDNA replication has recently become the subject of much debate. On the basis of ultrastructural and biochemical analyses, mtDNA replication had been considered to occur by a strand-displacement model[20], in which replication of the leading (heavy (H)) strand occurs first, initiating at a specific site (called O_H) in the non-coding control region (FIG. 1). When leading-strand replication has reached two-thirds around the genome, the origin of replication (O_L) on the lagging (light (L)) strand is exposed, allowing L-strand synthesis to occur in the opposite direction. Recent studies using two-dimensional gel electrophoresis challenge this model by providing evidence, in human, mouse and avian tissues, of a more conventional replication mechanism in which leading-lagging strand DNA replication is coordinated[21–23]. The two modes of replication have been hotly debated[24–26] and the recent description of another D-loop origin of replication adds further interest to this debate[27]. Nascent chains that start at this origin do not terminate prematurely and seem to be mainly responsible for mtDNA maintenance under steady-state conditions.

Our understanding of the transcriptional machinery in mammalian mitochondria has improved, predominantly owing to the identification of specific proteins and the development of an *in vitro* system to dissect out the regulatory features[28]. In human mtDNA, transcriptional initiation can occur on both strands. Transcription from the mitochondrial promoters produces a POLYCISTRONIC precursor RNA that is then processed to produce individual tRNA and mRNA molecules[29,30]. To initiate transcription, the dedicated mitochondrial RNA polymerase (POLRMT) requires TFAM, and either mitochondrial transcription factor B1 (TFB1M) or B2 (TFB2M)[31,32]. Recent evidence shows that TFAM induces a structural change of the light-strand promoter that is required for POLRMT-dependent promoter recognition[33]. The importance of mitochondrial transcription to cellular dysfunction as a result of pathogenic mtDNA mutations is a neglected area of research that might give important insights into some of the tissue-specific or mutation-specific effects.

The mRNAs for the 13 mtDNA-encoded OXPHOS proteins are translated on mitochondrial ribosomes. Mutations in mitochondrial tRNA or rRNA genes affect pathology by disrupting mitochondrial translation[34] and recent research has concentrated on identifying nuclear factors, including mitochondrial ribosomal proteins (MRPs), that are essential for this process. The first mutations in these nuclear genes, including *MRPS16* (mitochondrial ribosomal protein S16)[35] and *EFG1* (officially known as *GFM1*; G elongation factor, mitochondrial 1)[36] have recently been described in consanguineous families that have generalized mitochondrial translation defects.

Homoplasmy and heteroplasmy. The polyploid nature of the mitochondrial genome — up to several thousand copies per cell — gives rise to an important feature of mitochondrial genetics, homoplasmy and heteroplasmy. In simple terms, homoplasmy is when all copies of the mitochondrial genome are identical;

Table 1 | **Comparison between the human nuclear and mitochondrial genomes***

Characteristic	Nuclear genome	Mitochondrial genome
Size	~3.3 x 10^9 bp	16,569 bp
Number of DNA molecules per cell	23 in haploid cells; 46 in diploid cells	Several thousand copies per cell (polyploidy)
Number of genes encoded	~20,000–30,000	37 (13 polypeptides, 22 tRNAs and 2 rRNAs)
Gene density	~1 per 40,000 bp	1 per 450 bp
Introns	Frequently found in most genes	Absent
Percentage of coding DNA	~3%	~93%
Codon usage	The universal genetic code	AUA codes for methionine; TGA codes for tryptophan; AGA and AGG specify stop codons
Associated proteins	Nucleosome-associated histone proteins and non-histone proteins	No histones; but associated with several proteins (for example, TFAM) that form nucleoids
Mode of inheritance	Mendelian inheritance for autosomes and the X chromosome; paternal inheritance for the Y chromosome	Exclusively maternal
Replication	Strand-coupled mechanism that uses DNA polymerases α and δ	Strand-coupled and strand-displacement models; only uses DNA polymerase γ
Transcription	Most genes are transcribed individually	All genes on both strands are transcribed as large polycistrons
Recombination	Each pair of homologues recombines during the prophase of meiosis	There is evidence that recombination occurs at a cellular level but little evidence that it occurs at a population level

*Table modified from REF. 171 © (1999) John Wiley and Sons. TFAM, mitochondrial transcription factor A; rRNA, ribosomal RNA.

NUCLEOID
A dynamic complex that consists of several copies of mitochondrial DNA and key maintenance proteins within the organelle.

POLYCISTRONIC
A form of gene organization that results in transcription of an mRNA that codes for multiple gene products, each of which is independently translated from the mRNA.

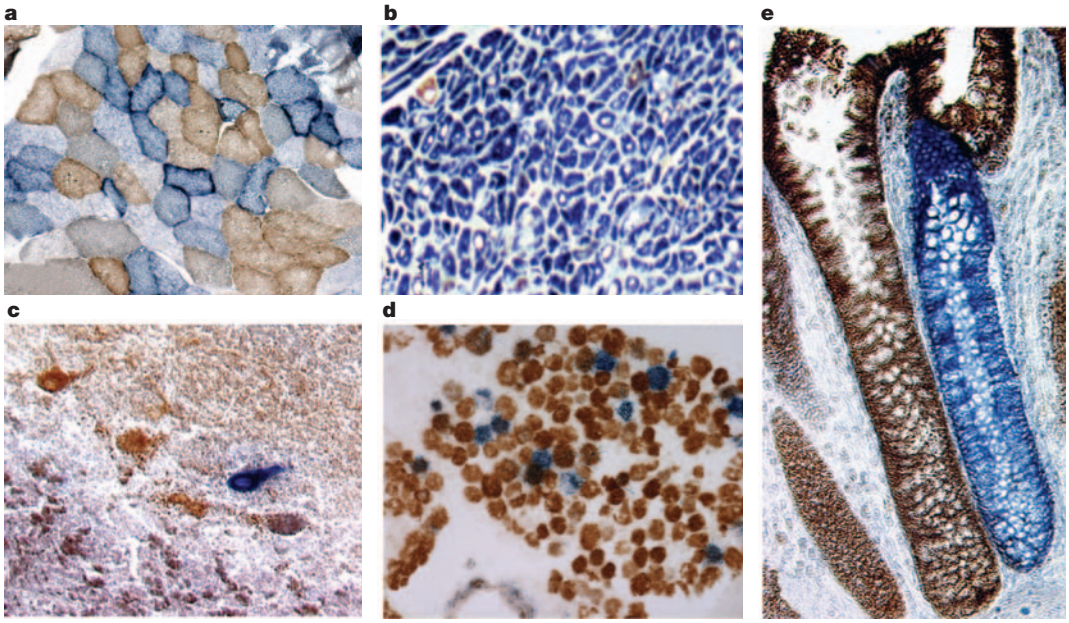

Figure 2 | **Cytochrome *c* oxidase deficiency in mitochondrial DNA-associated disease and ageing.** Transverse tissue sections that are reacted for both cytochrome *c* oxidase (COX) and succinate dehydrogenase (SDH) activities sequentially, with COX-positive cells shown in brown and COX-deficient cells shown in blue. **a** | Skeletal muscle from a patient with a heteroplasmic mitochondrial tRNA point mutation. The section shows a typical 'mosaic' pattern of COX activity, with many muscle fibres harbouring levels of mutated mtDNA that are above the crucial threshold to produce a functional enzyme complex. **b** | Cardiac tissue (left ventricle) from a patient with a homoplasmic tRNA mutation that causes hypertrophic cardiomyopathy, which demonstrates an absence of COX in most cells. **c** | A section of cerebellum from a patient with an mtDNA rearrangement that highlights the presence of COX-deficient neurons. **d**,**e** | Tissues that show COX deficiency that is due to clonal expansion of somatic mtDNA mutations within single cells — a phenomenon that is seen in both post-mitotic cells (**d**; extraocular muscles) and rapidly dividing cells (**e**; colonic crypt) in ageing humans.

heteroplasmy is when there is a mixture of two or more mitochondrial genotypes. The value of these terms is apparent when we consider mtDNA mutations that lead to disease. Some mutations affect all copies of the mitochondrial genome (homoplasmic mutation), whereas others are only present in some copies of the mitochondrial genome (heteroplasmic mutation). In the presence of heteroplasmy, there is a threshold level of mutation that is important for both the clinical expression of the disease and for biochemical defects, as routinely demonstrated by the cytochemical assessment of cytochrome *c* oxidase (COX; complex IV) activity in an individual cell (FIG. 2). Numerous single-cell and *TRANS*MITOCHONDRIAL CYBRID CELL studies have shown that the mutated form is functionally recessive and that a biochemical phenotype is associated with high levels of mutation above a crucial threshold[37].

The concept of homoplasmy is more apparent than real. In most individuals there is no evidence of heteroplasmy, but all available evidence indicates that mtDNA is constantly undergoing mutation, with clonal expansion or loss of either point mutations or deletions[38,39]. Because these acquired mutations occur at random, all acquired mutations will be present at a low level and therefore might not be detected in a tissue homogenate or blood sample. Perhaps we should only use 'homoplasmy' to describe a state in which we cannot detect these acquired mutations, although it would be naive to think that all mitochondrial genomes within an individual, tissue or even a cell are identical.

Inheritance of mitochondrial DNA. The standard model of mtDNA inheritance is that it is transmitted strictly through the maternal line[40] and that mtDNA lineages are therefore clonal. However, this model has recently been challenged. Low levels of paternal transmission of mtDNA have been observed in crosses between mouse species, but not within species[41], although further studies showed that this paternal mtDNA was not transmitted to the subsequent generation[42]. In addition, there is evidence that recombination has contributed to the distribution of mtDNA polymorphisms within the human population[43]. Although recombination might occur at the cellular level[44,45], the occurrence of recombination within the population is contentious[46]. Studies of a patient with mitochondrial myopathy — who carried a novel, 2-bp pathogenic deletion in the NADH (reduced nicotinamide adenine dinucleotide) dehydrogenase subunit 2 (*ND2*) gene in his muscle mtDNA — have also challenged the maternal inheritance of mtDNA[47]. Using haplotype analysis, this sporadic mutation was present in paternally derived mtDNA, although the deletion itself was not evident in the father's mtDNA. With the exception of skeletal muscle, all other tissues contained a single, maternally derived mtDNA haplotype. Subsequent studies of other patients

*TRANS*MITOCHONDRIAL CYBRID
Derived from the term 'cytoplasmic hybrid'. A cell line that is used to study the pathophysiological consequences of any given mitochondrial DNA mutation in a control nuclear background. Cybrids are generated by fusing patient cytoplasts (enucleated cells that contain mitochondria) with a cell line that lacks mitochondrial DNA.

with mitochondrial myopathies have not shown any evidence of paternal transmission[48–50], even when assisted reproduction techniques were applied[51,52].

Mitochondrial DNA transmission. The current genetic advice is that fathers with mtDNA mutations are at no risk of transmitting the defect to their offspring. Maternal transmission of mutated mtDNA occurs, but the risk depends on the type of mutation and possibly the segregation of the mutation within maternal tissues. Identifying specific mtDNA mutations and investigating family members for evidence of transmission will give guidance to the likelihood of transmission through the germline. Detailed studies of large patient cohorts provide invaluable information on the risk of transmission. A recent analysis of a single, large-scale mtDNA deletion in 226 families, showed that the risk of recurrence in the offspring of an affected mother was 4.11% (REF. 53). If the mother was unaffected, then there was no record of affected siblings, which indicates that the risk of recurrence is negligible.

Homoplasmic mtDNA mutations are transmitted to all maternal offspring. However, the genetic advice given to these families is not straightforward. Most patients with LEBER HEREDITARY OPTIC NEUROPATHY (LHON; see also Online links box) have homoplasmic mtDNA mutations. Although all offspring inherit the mutation, only some will develop the disease. Approximately 50% of males, but only 10% of females, develop impaired vision[54], which certainly indicates that nuclear genetic factors are important in the expression of the disease. Another homoplasmic mtDNA mutation, the 1555A>G 12*S* ribosomal RNA (*RNR1*) mutation[55], is an important cause of post-lingual deafness. Its clinical expression depends on the administration of aminoglycoside antibiotics, which highlights the importance of environmental factors in the expression of homoplasmic mtDNA disease. Although it is obvious that genetic counselling has to be tailored to specific mutations, further information is required to determine the risk for individual defects.

The transmission of heteroplasmic mtDNA point mutations is even more complex. Nuclear genetic[56] and environmental factors almost certainly affect expression of the disease phenotype. In addition, there is a GENETIC BOTTLENECK during development, and the amount of mutated mtDNA that is transmitted to the offspring is variable[57] (FIG. 3). Because many of the clinical features depend on the relative proportions of mutated versus wild-type mtDNA, the outcome for each pregnancy remains difficult to predict[58].

Clinical features of human mtDNA disease

Mitochondria are vital components of all nucleated cells. Therefore, it is not surprising that mtDNA diseases affect many tissues and that the clinical features

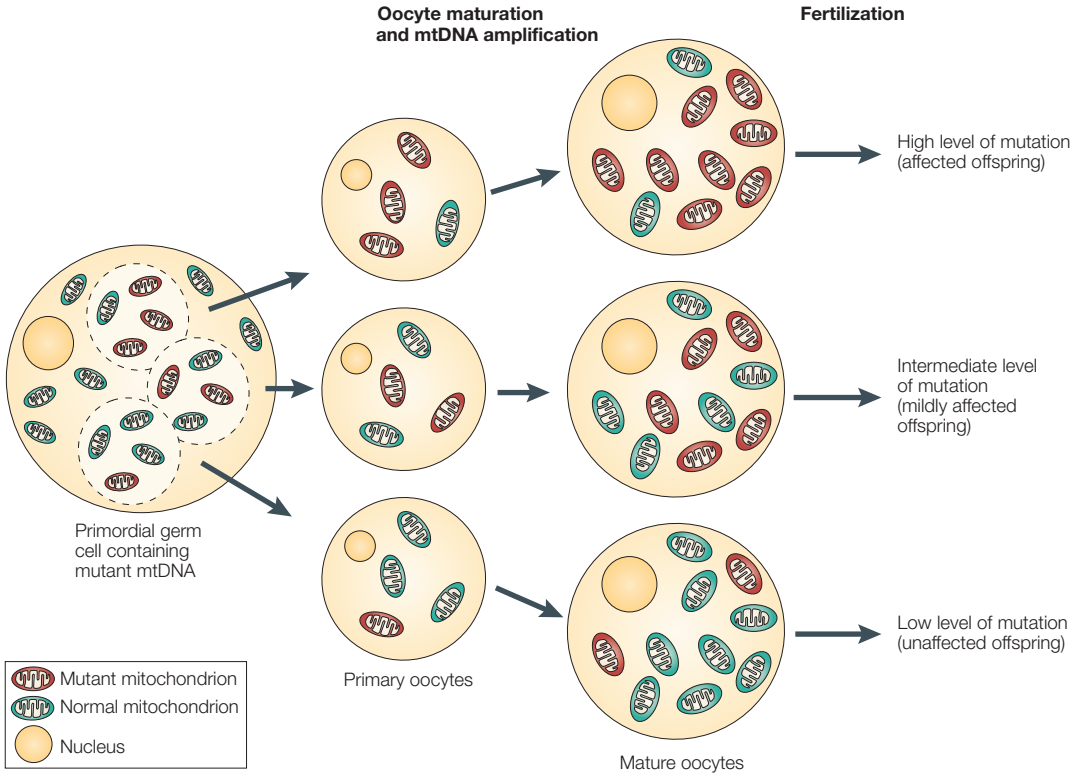

Figure 3 | **The mitochondrial genetic bottleneck.** During the production of primary oocytes, a selected number of mitochondrial DNA (mtDNA) molecules are transferred into each oocyte. Oocyte maturation is associated with the rapid replication of this mtDNA population. This restriction-amplification event can lead to a random shift of mtDNA mutational load between generations and is responsible for the variable levels of mutated mtDNA observed in affected offspring from mothers with pathogenic mtDNA mutations. Mitochondria that contain mutated mtDNA are shown in red, those with normal mtDNA are shown in green.

LEBER HEREDITARY OPTIC NEUROPATHY
A mitochondrial disease that is characterized by optic nerve dysfunction, which leads to bilateral visual failure in young adults.

GENETIC BOTTLENECK
A temporary reduction in population size that causes the loss of genetic variation.

Box 1 | Common features of mitochondrial DNA-associated diseases

Adult

- **Neurological: migraine | strokes | epilepsy | dementia | myopathy | peripheral neuropathy |** DIPLOPIA **|** ATAXIA **| speech disturbances | sensorineural deafness**
- **Gastrointestinal: constipation | irritable bowel |** DYSPHAGIA
- **Cardiac: heart failure | heart block | cardiomyopathy**
- **Respiratory: respiratory failure | nocturnal hypoventilation | recurrent aspiration | pneumonia**
- **Endocrinal: diabetes | thyroid disease | parathyroid disease | ovarian failure**
- **Ophthalmological: optic atrophy | cataract | ophthalmoplegia |** PTOSIS

Paediatric

- **Neurological: epilepsy | myopathy | psychomotor retardation | ataxia | spasticity |** DYSTONIA **| sensorineural deafness**
- **Gastrointestinal: vomiting | failure to thrive | dysphagia**
- **Cardiac: biventricular hypertrophic cardiomyopathy | rhythm abnormalities**
- **Respiratory: central hypoventilation | apnoea**
- **Haematological: anaemia |** PANCYTOPAENIA
- **Renal: renal tubular defects**
- **Liver: hepatic failure**
- **Endocrinal: diabetes | adrenal failure**
- **Ophthalmological: optic atrophy**

are so variable (BOX 1). Such diversity makes it difficult to define the impact of mtDNA mutations on human health, however, stratifying the clinical features into the following groups defines the extent of the problem: classic mtDNA syndromes, clinical syndromes with a high risk of mtDNA mutations, involvement in common disease phenotypes and mtDNA as a predisposition for common disease and ageing.

Clinical features are specific and clinical diagnosis is straightforward for patients with the classic syndromes. However, in many patients, the clinical symptoms might be non-specific and mtDNA disease is only one of several possible diagnoses. Important clues to a possible mitochondrial origin for the symptoms include a maternal family history and the involvement of several tissues (BOX 2). For a large number of patients, diagnosis depends on the results of several lines of investigation. This includes histochemical and biochemical studies to determine the precise nature of the respiratory chain defect, analysis of the mitochondrial genome for common mutations and, finally, complete genome sequencing to look for rare or novel mutations[59]. The mitochondrial genome is highly variable and the ease of whole-genome sequencing does not resolve some diagnostic dilemmas because the interpretation of a novel sequence change can be difficult in relation to potential pathogenicity[60]. Accurate diagnosis of an mtDNA defect is essential because of the consequences for both the patient and the family members.

Classic syndromes. Genetic defects of the human mitochondrial genome were first described in 1988 (REFS 61,62) and arose from the investigation of two syndromes — Kearns–Sayre syndrome (KSS) and LHON. In KSS, single, large-scale mtDNA deletions (usually sporadic) were detected in muscle biopsies, and mitochondrial ultrastructural and cytochemical abnormalities in the muscle were apparent. For LHON, a strict maternal pattern of inheritance was evident and point mutations involving the ND family of genes that encode subunits of complex I were identified. Since 1988, several mutations[63] of the mitochondrial genome have been identified and associated with disease (TABLE 2).

Clinical syndromes that have a high probability of mitochondrial DNA involvement. Over the past decade, we have become aware of several clinical syndromes that might be associated with mtDNA mutations. The increasing recognition of mtDNA involvement in disease is partially due to the relative ease of sequencing the mitochondrial genome, although defining pathogenicity of specific base substitutions can be difficult[60]. Examples of such diseases include progressive external OPHTHALMOPLEGIA[64], PEARSON SYNDROME[65], LEIGH SYNDROME[66,67] (see also Online links box), exercise-induced muscle pain, fatigue and RHABDOMYOLYSIS[68] (see also Online links box), and amino-glycoside-induced hearing loss[55]. For some of these conditions, such as progressive external ophthalmoplegia, mtDNA disease is the most likely cause, whereas in others, such as Leigh syndrome, there is a long list of potential genetic causes.

Involvement in common disease phenotypes. The principal difficulty for clinicians is that patients with mtDNA disease rarely have a classic phenotype and mtDNA enters into the differential diagnosis of many

DIPLOPIA
Double vision; derived from the Greek *diplous*, meaning double, and *ops*, meaning eye.

ATAXIA
The loss of the ability to coordinate muscular movement.

DYSPHAGIA
A difficulty in swallowing.

PTOSIS
The abnormal lowering or drooping of the upper eyelid that is caused by muscle weakness.

DYSTONIA
A neurological movement disorder that is characterized by involuntary muscle contractions that might cause twisting or jerking movements of the body.

PANCYTOPAENIA
A deficiency of all blood cells including red cells, white cells and platelets.

OPHTHALMOPLEGIA
Weakness of one or more of the muscles that control eye movement.

PEARSON SYNDROME
A severe disease during infancy that affects bone marrow and pancreas function owing to large-scale rearrangements of the mitochondrial genome.

Box 2 | Guidelines for the recognition of patients with mitochondrial DNA disease

A list of the clinical guidelines that have been generated from our experience of investigating and managing patients with mtDNA mutations.

- **Maternal inheritance**
- **Recognition of classic syndromes; for example, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) or chronic progressive external ophthalmoplegia (CPEO)**
- **Recognition of characteristic clinical features; for example, biventricular cardiac hypertrophy**
- **Involvement of many organ systems; for example, diabetes and deafness**
- **Specific combinations of symptoms; for example, strokes and migraine and seizures and ataxia**
- **Abnormal clinical or laboratory investigations; for example, lactic acidaemia in children, characteristic magnetic-resonance-imaging findings, abnormal muscle biopsy that is associated with** RAGGED-RED FIBRES **or cytochrome *c* oxidase-deficient fibres**

Table 2 | **Clinical disorders that are caused by mutations in mitochondrial DNA**

Mitochondrial DNA disorder	Clinical phenotype	mtDNA genotype	Gene	Status	Inheritance	Reference
Kearns–Sayre syndrome	Progressive myopathy, ophthalmoplegia, cardiomyopathy	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic	61,158
CPEO	Ophthalmoplegia	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic	61, 64
Pearson syndrome	Pancytopoenia, lactic acidosis	A single, large-scale deletion	Several deleted genes	Heteroplasmic	Usually sporadic	65
MELAS	Myopathy, encephalopathy lactic acidosis, stroke-like episodes	3243A>G; 3271T>C Individual mutations	*TRNL1* *ND1 and ND5*	Heteroplasmic Heteroplasmic	Maternal Maternal	159 160, 161
MERRF	Myoclonic epilepsy, myopathy	8344A>G; 8356T>C	*TRNK*	Heteroplasmic	Maternal	162
NARP	Neuropathy, ataxia, retinitis pigmentosa	8993T>G	*ATP6*	Heteroplasmic	Maternal	163
MILS	Progressive brain-stem disorder	8993T>C	*ATP6*	Heteroplasmic	Maternal	67
MIDD	Diabetes, deafness	3243A>G	*TRNL1*	Heteroplasmic	Maternal	164
LHON	Optic neuropathy	3460G>A 11778G>A 14484T>C	*ND1* *ND4* *ND6*	Hetero- or homoplasmic Hetero- or homoplasmic Hetero- or homoplasmic	Maternal Maternal Maternal	165 62 166
Myopathy and diabetes	Myopathy, weakness, diabetes	14709T>C	*TRNE*	Hetero- or homoplasmic	Maternal	167,168
Sensorineural hearing loss	Deafness	1555A>G Individual mutations	*RNR1* *TRNS1*	Homoplasmic Hetero- or homoplasmic	Maternal Maternal	55 169,170
Exercise intolerance	Fatigue, muscle weakness	Individual mutations	*CYB*	Heteroplasmic	Sporadic	68
Fatal, infantile encephalopathy; Leigh/Leigh-like syndrome	Encephalopathy, lactic acidosis	10158T>C; 10191T>C	*ND3*	Heteroplasmic	Sporadic	66

ATP6, ATPase 6; CPEO, chronic progressive external ophthalmoplegia; *CYB*, cytochrome *b*; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy and ragged-red fibres; MIDD, maternally-inherited diabetes and deafness; MILS, maternally-inherited Leigh syndrome; *ND1,3–6*, NADH dehydrogenase subunits 1,3–6; NARP, neurogenic weakness, ataxia and retinitis pigmentosa; *RNR1*, 12*S* ribosomal RNA; *TRNE, TRNK, TRNL1, TRNS1*, mitochondrial tRNAs.

LEIGH SYNDROME
A disease that affects the brainstem and basal ganglia and is characterised by defects in mitochondrial oxidative phosphorylation.

RHABDOMYOLYSIS
The breakdown of muscle fibres owing to injury, toxins or metabolic disease, which leads to high concentrations of myoglobin in both plasma and urine

RAGGED-RED FIBRES
The pathological hallmark of mtDNA disease that is characterized by the subsarcolemmal accumulation of abnormal mitochondria in the muscle fibre, which stains red with a Gomori trichrome stain.

common clinical syndromes. A good example of a common condition in which mtDNA is a potential cause is diabetes, the most common metabolic disease to affect humans[69]. mtDNA disease certainly is associated with diabetes, but these patients represent a tiny proportion of those affected with diabetes[70–72]. Because several mtDNA mutations cause diabetes[63] it is clearly impractical and uneconomical to screen all patients with diabetes for causative mutations. From the list of common clinical features seen in patients with mtDNA disease (BOX 1), it is obvious that it is difficult to decide which patients to investigate for possible mtDNA disease. Further difficulties in this area have been highlighted by a recent report that describes a clear association between members of a large family that harbour a homoplasmic mitochondrial tRNA mutation and a metabolic syndrome that is characterized by hypertension, hypomagnesaemia and hypercholesterolaemia[9]. Because conditions such as hypertension and hypercholesterolaemia affect many individuals[73], the number of patients with mtDNA disease is potentially very high. However, it must be kept in the context that most reports describe isolated families. In epidemiological surveys of populations[74], the incidence of mtDNA mutations within a specific disease phenotype[75] is small. Other factors that make the diagnosis of mtDNA difficult include the specific combination of clinical symptoms (BOX 2); for example, mitochondrial diabetes is often accompanied by deafness[70], and stroke-like episodes are usually associated with migraine-like symptoms. There is no doubt that many patients with mtDNA mutations go undiagnosed for years and many are probably never recognized. Increased awareness among physicians and paediatricians is important, as is the investigation of patients in specialist centres that are able to provide a complete range of diagnostic tests.

Mitochondrial DNA variants as a predisposition for common disease. There has been considerable interest in the possibility that mtDNA variants might predispose

to common diseases; for example, diabetes, Alzheimer disease (AD) and Parkinson disease (PD)[76]. The known association of diabetes with mtDNA mutations and the involvement of reactive oxygen species in the aetiology of AD and PD has encouraged a large number of studies. The first approach involves identifying candidate pathogenic mutations by comparing mtDNA sequences between patients and control groups. Unfortunately, interpreting the results is limited by the relatively small number of individual mtDNA sequences in each group and by the high mutation rate of mtDNA, which results in a high frequency of rare variants or private polymorphisms[77]. Some variants are more common than others. For example, the 16189 variant, a variable length polycytosine tract in the non-coding control region, has been associated with several late-onset, multifactorial disorders, including susceptibility to cardiomyopathy[78] and type 2 diabetes[79], and has been proposed to affect mtDNA replication[79]. However, because this variant occurs frequently within the population, concerns arise (that are similar to the 'haplogroup' studies) over the control cohorts that are chosen for such population-based surveys. Human populations can be divided into several mtDNA haplogroups that are based on specific SNPs, reflecting mutations accumulated by a discrete maternal lineage[80]. Therefore, haplogroup association studies have been used to define the role of mtDNA mutations in complex diseases. In this approach, disease and control mtDNA sequence sets are assigned to haplogroups, and the distribution of haplogroups is compared. A significant difference is interpreted to indicate that the mtDNA 'background' has an effect on expression or penetrance of the clinical abnormalities. Various results have been reported for both AD and PD[81–84]. Given the inconsistency among different studies, it is difficult to conclude unequivocally that the risk of either PD or AD is influenced by mitochondrial haplogroups. Some of the inconsistency could be due to population-substructure differences between disease and control groups. It is difficult to 'match' groups, even when one tries to control nationality and ethnicity[85].

Models of mitochondrial DNA disease

The inability to genetically engineer or transform the mitochondrial genome *in vitro* or *in vivo* has severely hampered efforts to study the relationship between mtDNA genotype and clinical phenotype in animal models that bear heteroplasmic, pathogenic mtDNA mutations. Despite this, much has been learned of the biochemical and cellular consequences of specific mtDNA mutations through the generation of *trans*mitochondrial cytoplasmic cybrid cells. In this system, immortalized human cell lines that have been completely depleted of their endogenous mtDNA (ρ° cells) are repopulated with exogenous, patient mitochondria. As ρ° cells have no functional respiratory chain and are dependent on pyruvate and uridine for growth, the loss of either of these two metabolic requirements can be used to select for transformants that harbour complementing (exogenous) mtDNA[86]. This elegant system allows the functional and physiological consequences of different levels of heteroplasmy of certain mtDNA mutations to be tested[87,88]. In addition, it has also proved useful in determining the genetic origin of certain mitochondrial disorders[89,90], the effect of nuclear background on the segregation of pathogenic mtDNA mutations[91] and in identifying the first tRNA suppressor mutation in human mitochondria[92]. It has also been used to test rational, genetic therapies and more recently, to demonstrate the presence of heterologous mtDNA recombination in human cells[44].

The generation of heteroplasmic mice that harbour pathogenic mtDNA mutations was frustratingly difficult. Heteroplasmic mice were first generated by fusing zygotes that carried one mtDNA haplotype with enucleated embryos that carried a different haplotype[93,94], although these mice (BALB and NZB) harboured only neutral mtDNA variants. Further advances were made with the generation of chimeric, heteroplasmic chloramphenicol resistant (CAP^R) mice, which could transmit mtDNA mutations to subsequent generations and showed signs of mitochondrial dysfunction[95,96]. By fusing CYTOPLASTS that harbour high levels of a somatic mtDNA rearrangement to one-cell embryos, Hayashi and colleagues generated a model that has a pathogenic deletion or duplication[97] of the mitochondrial genome, which was transmitted through the germ line. These mice showed muscle and cardiac defects, but also, more prominently, renal failure together with lactic acidosis, anaemia and a mosaic distribution of COX deficiency. Human mtDNA-rearrangement mutations rarely result in renal disease, so this experiment highlighted the potential for species-specific symptoms and signs. Although subsequent studies that use this mouse model have improved our understanding of mitochondrial segregation and disease pathogenesis[98], no reported studies specifically address the development of potential therapies.

The generation of mouse models that target nuclear genes involved in mtDNA maintenance or replication, such as the *Tfam* knockout mouse, has proved to be a more successful approach[99–103]. Using the Cre-*loxP* recombination system, Larsson and colleagues disrupted the *Tfam* gene in selected mouse tissues, yielding models that provide clues to potentially important factors in the development of specific clinical features associated with mtDNA disease. For example, the mice generated by the postnatal disruption of *Tfam* in neurons of the hippocampus and neocortex develop a late-onset neurodegeneration, but there is minimal cell loss until the mice developed seizures[101]. This finding indicates that stress or neuronal discharge resulted in an acute energy crisis, which highlights the importance of management issues such as the effective control of seizures in patients with mtDNA disease.

Management of mtDNA diseases

Although defining the clinical features and establishing the diagnosis of mtDNA disease remain considerable challenges, the development of effective curative treatment is also a challenging hurdle for experimental

CYTOPLAST
An enucleated donor cell that contains patient mitochondria and that is used to generate cybrid fusions.

scientists. However, there are important issues associated with the clinical management of patients and many of these issues have recently been discussed[104]. Early detection of treatable symptoms is crucial.

Mitochondrial DNA mutation levels can be manipulated through exercise. In healthy individuals, lack of exercise (deconditioning) leads to an overall reduction in mitochondrial enzyme activity, whereas endurance training improves enzyme activity. Resistance training or muscle necrosis stimulates the incorporation of SATELLITE CELLS into existing muscle fibres[105,106]. It is postulated that for sporadic mutations, resistance training might lead to an overall reduction in the proportion of mutated mtDNA versus wild-type, as satellite cells contain a low or negligible amount of mutated mtDNA[106,107]. Endurance training might therefore improve function by increasing wild-type mtDNA levels. However, there are concerns that mutated mtDNA might be preferentially amplified, and that this increase might become clinically relevant after deconditioning[105,108]. Studies are currently underway to address both the improvements and the concerns arising from these earlier reports.

New avenues for treatment of mitochondrial diseases. The polyploid nature of the mitochondrial genome, the phenomenon of heteroplasmy, the inability to deliver potentially therapeutic nucleic acids to the organelle through mitochondrial transfection, and the slow realization of appropriate animal models of mtDNA disease are the main problems for the development of gene-based treatments. Despite these, several strategies that seek to rescue mitochondrial function through complementation of the genetic defect or direct manipulation of the levels of mutated mtDNA have been considered.

One way of complementing a dysfunctional mitochondrial protein is through ALLOTOPIC EXPRESSION. Using this approach, it was possible to express the wild-type ATPase 6 protein allotopically from nucleus-transfected constructs in *trans*mitochondrial cybrid cells that were homoplasmic for the 8993T>G *MTATP6* (subunit 6 of mitochondrial ATP synthase) mutation (which causes neurogenic weakness, ataxia and retinitis pigmentosa (NARP) syndrome) and demonstrate a partial rescue of the biochemical defect[109]. A similar strategy has been used to express a modified NADH dehydrogenase subunit 4 (*ND4*) gene to complement the 11778G>A mutation that causes LHON[110]. To complement mitochondrial tRNA defects, an alternative approach has been proposed. Yeast cytosolic $tRNA^{LysCUU}$ (tK1) and similar derivatives can be imported into isolated human mitochondria if the tRNA is amino-acylated and supplied with soluble factors, including lysl-tRNA synthetases[111]. Using *trans*mitochondrial cybrid cells and primary human fibroblasts that carry the 8344A>G *TRNK* (a tRNA gene) mutation, which causes myoclonic epilepsy and ragged-red fibres (MERRF) syndrome, it has recently been shown that the imported $tRNA^{Lys}$ is correctly amino-acylated and able to participate in mitochondrial translation, partially rescuing mitochondrial function[112].

Because the recessive nature of many pathogenic mutations dictates that a small percentage of the wild-type genotype is protective, a minor adjustment to the balance of mutated to wild-type genomes in favour of the wild type could improve both biochemical function and resolve the clinical problem. The selective inhibition of mutated mtDNA replication by antigenomic agents has been applied to manipulate mtDNA heteroplasmy[113]. Another strategy involves targeting the organelle with restriction endonucleases that, by differentiating between mtDNA genotypes, can cause the preferential elimination of the mutated genotype and propagation of the wild-type genotype[114,115]. Pharmacological approaches to shift mtDNA heteroplasmy are also being investigated. Oligomycin, an irreversible inhibitor of mitochondrial ATP synthase, has been used to increase the fraction of wild-type molecules in cells that harbour the 8933T>G *MTATP6* mutation under culture conditions (using galactose as the sole carbon source) that specifically select for the wild-type molecule[116]. This culture medium could also selectively kill cells that are homoplasmic for a common (4,977-bp deletion) mtDNA rearrangement — the molecular abnormality in many patients with KSS. In this case, a ketogenic medium has been used to shift the heteroplasmy of cells that contain a mixture of wild-type and partially deleted mtDNAs[117]. Finally, the revelation that overexpressing mitochondrial Tu translation elongation factor, TUFM, in yeast mutant counterparts of *TRNL1* (a tRNA gene) MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) mutations facilitates the rescue of the respiratory defect might have implications for human disease-related mutations[118].

Prevention of transmission of mtDNA disease

Improving genetic advice. The question often asked by both families and clinicians is whether there are any prospects for providing either better guidance for families or even preventing the transmission of mtDNA disease. AMNIOCENTESIS and CHORIONIC VILLUS BIOPSY are widely used to diagnose autosomal and chromosomal abnormalities, and might be of value in heteroplasmic mtDNA disorders. Chorionic villus biopsy has been used in some families that have heteroplasmic mtDNA disorders, where the results have influenced the clinical management[119–121]. In many heteroplasmic mtDNA[64,122] disorders there are marked tissue-specific differences in the level of heteroplasmy, and therefore a reasonable concern is whether a prenatal sample will reflect the likely outcome for the fetus. The present evidence indicates that this should not be a problem[94,123]. Preimplantation genetic diagnosis (PGD) allows either analysis of mtDNA from the POLAR BODY of unfertilized oocytes, or from one to two single cells that are taken from 8-cell embryos, followed by the implantation of healthy embryos. The potential value of this technique is obvious, but there is little reported information of its application in patients with mtDNA mutations.

SATELLITE CELLS
Quiescent cells that are located between the basal lamina and the plasmalemma of the muscle fibre, and are a main contributor to postnatal muscle growth.

ALLOTOPIC EXPRESSION
The expression of a gene in a different cellular compartment to its target location. In the context described here, it is the recoding of a mitochondrial gene to allow it to be expressed in the nucleus. The subsequent conjugation of a mitochondrial-targeting sequence promotes import and localization of the gene product to the organelle.

AMNIOCENTESIS
An aspiration of cells from the amniotic sac, using a needle, for biochemical and genetic analysis.

CHORIONIC VILLUS BIOPSY
A placental biopsy that is carried out in early pregnancy to collect fetal tissue for genetic and biochemical analysis.

POLAR BODY
A small haploid cell that is produced during oogenesis and that does not develop into a functional ovum.

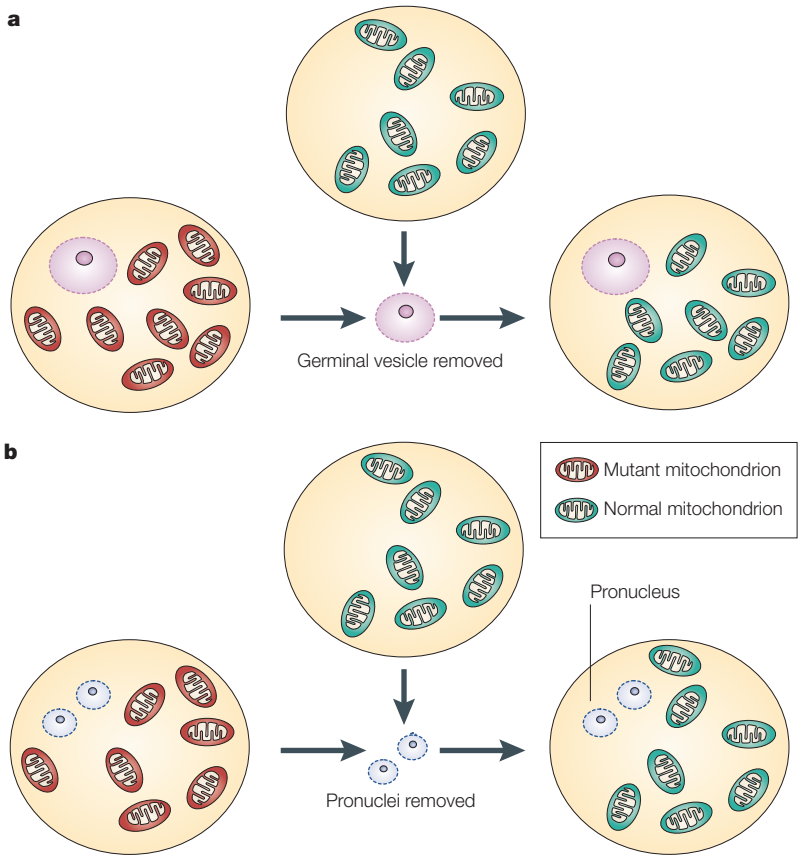

Figure 4 | **Nuclear transfer techniques.** The prevention of mutated maternal mtDNA transmission could be achieved by nuclear transfer techniques. In theory, this could be carried out at the germinal vesicle stage (**a**), when clearly identifiable structures are visible. The germinal vesicle contains nuclear chromosomes and could be transferred to an oocyte that contains normal mtDNA (green). Alternatively, pronuclear transfer can be carried out at the fertilized oocyte stage (**b**); this involves removal of the male and female pronuclei from the oocyte that contains mutated mtDNA (red) and their subsequent transfer to an oocyte that has normal mtDNA (green).

The high number of mitochondrial genomes within oocytes[124] indicates that PGD should be feasible for mtDNA disease. Experiments in heteroplasmic mice have shown the levels of heteroplasmy to be virtually identical between the ooplasm and polar body of the mature oocyte and also between blastomeres of each of the 2-, 4-, and 6–8-cell embryo[125].

Alternative strategies associated with oocyte manipulation. Development of new strategies to prevent the transmission of mtDNA is a very important aspect of disease management. An obvious method to prevent the transmission of an mtDNA mutation to the offspring is oocyte donation. However, although the oocyte would be fertilized using the father's sperm, the child would have the nuclear genotype of the donor female, a less-than-ideal option for many mothers. Cytoplasmic transfer involves the transfer of normal mitochondria into the abnormal oocyte to dilute the effect of any mtDNA defect[126]. Cytoplasmic transfer between human oocytes has been carried out to improve the outcome of assisted reproduction methods[127]. The effectiveness of this procedure is uncertain[128], but some of the children born were heteroplasmic with low levels of mtDNA from the donor oocyte[129]. However, despite the observation that changes in heteroplasmy can occur, it is likely that this technique will have limited value for patients with mtDNA disease, as mouse experiments indicate that the amount of mtDNA that can be transferred is relatively small[130].

Alternatively, nuclear chromosomes from an oocyte that contains mutated mtDNA could be transferred to an enucleated oocyte from a normal female[131]. This method has obvious advantages: the offspring will be genetically related to both parents and the chances of achieving very low levels of mutated mtDNA in the oocyte are high. However, important technical and ethical or legal issues make progress in this area difficult, principally because the technique involves the genetic manipulation of fertilized oocytes. To overcome these issues, the transfer could be carried out at different stages of oocyte maturation, although transfer from mature unfertilized oocytes is difficult because of the serious risk of aneuploidy that is due to chromosome loss during transfer or due to missegregation following the disruption of the meiotic spindle. Karyoplast transfer of the GERMINAL VESICLE between oocytes might be possible before *in vitro* maturation and subsequent fertilization (FIG. 4). However, although maturation and fertilization of normal germinal-vesicle-stage oocytes has been achieved with both mouse[132–134] and human oocytes[135,136], these methods are very inefficient. Pronuclear-stage transfer is possibly more feasible because human pronuclear oocytes can be manipulated to generate normal offspring[137] and because this technique is feasible in the mouse[138,139].

GERMINAL VESICLE
A stage during oocyte maturation in which the oocyte nucleus is located close to the surface of the egg cell and is clearly visible.

Acquired mtDNA mutations in ageing and cancer

Mitochondrial theory of ageing. The mitochondrial theory of ageing proposes that progressive accumulation of somatic mutations in mtDNA during a lifetime leads to an inevitable decline in mitochondrial function[140]. The most important biochemical factors in this process are considered to be reactive oxygen species (ROS), which are generated at very low levels during the normal function of the mitochondrial respiratory chain. It has been proposed that ROS can cause somatic mutations in mtDNA[140]. These mutations then result in impaired function of the respiratory chain, leading to increased ROS production and the subsequent accumulation of more mutations. There is evidence that some pathogenic mutations, such as at 8993T>G, lead to increased ROS production[141]; interestingly, for 8993T>G, free radical-mediated inhibition of OXPHOS can be restored by antioxidants[142]. The ROS vicious cycle is believed to account for an exponential increase in oxidative damage during ageing, which results in the eventual loss of cellular and tissue functions through a combination of energy insufficiency, signalling defects, apoptosis and replicative senescence.

Studies in human and mouse models. Although the mitochondrial theory of ageing was first suggested in the 1950s, the first convincing evidence of a

GENETIC DRIFT
Changes in the frequency of a genetic variant in a population owing to chance alone.

biochemical defect in humans was the detection of COX-deficient cells in ageing post-mitotic tissues[143] (FIG. 2). These cells had identical characteristics to those found in patients with inherited mitochondrial disease[37]. Surprisingly, these cells show the clonal expansion of an individual mutation, which is often an mtDNA rearrangement, rather than many individual mtDNA mutations[144,145]. In addition, studies from patients showed that, in the presence of many mtDNA genomes within a cell, the mutation is functionally recessive and a threshold effect is observed[37]. More recently, mtDNA mutations were found to accumulate to high levels in dividing cells and presumably stem cells[39,146], and cause an observable biochemical defect[39].

How a mutation in an individual genome can clonally expand to high levels in individual cells is a crucial question. So far, several theories have been proposed, including the selective replication of mutated mtDNA, the longevity of mitochondria that contain mutated mtDNA and the 'sick mitochondrion' hypothesis (with preferential replication of mitochondria that have impaired OXPHOS activities and higher mutant load)[147–149]. However, mathematical-modelling studies have also indicated that this process might occur by GENETIC DRIFT[150].

Studies from ageing humans and animal models showed good correlation between ageing and the presence of mtDNA mutations. To test the mitochondrial ageing hypothesis, knock-in mice expressing a proof-reading-deficient version of PolgA were developed[10]. These mice acquire mtDNA mutations at a higher rate than is normal because the introduction of a point mutation into the exonuclease region of the *PolgA* gene drastically reduces its proof-reading exonuclease activity. The phenotype of the *PolgA* mutator mouse has many hallmarks of premature ageing, including a characteristic hunched appearance that is indicative of osteoporosis, reduced activity, severe weight loss with virtually no remaining adipose tissue and a marked reduction in muscle mass. Although this study provides strong evidence that somatic mtDNA mutations contribute to ageing, further experiments are required to confirm that somatic mtDNA mutations are causal in the ageing process.

Mitochondrial DNA mutations and cancer. In a landmark paper in 1998, Vogelstein and colleagues reported that mtDNA mutations were present in 7 out of 10 colorectal cancer cell lines that were studied[151]. Although these mtDNA mutations were somatic and absent in other patient tissues, many were homoplasmic in the tumour tissue. Since then, numerous other studies have documented the presence of somatic mtDNA mutations in both solid tumours and leukaemias[152–156]. How these mutations accumulate to high levels in individual tumours is still unclear, although both modelling and molecular studies indicate that they possibly accumulate without selection[38,39]. In addition, there is no evidence of whether mtDNA mutations themselves contribute to the development of the tumour. Original work on colorectal tumours showed that many of the mutations detected were not associated with a mitochondrial defect. Moreover, some of the mutations seen in other tumours are recognized polymorphic variants[151]. Therefore, although a direct link between the presence of the mtDNA mutation and the development of the tumour has not been made, the presence of a mtDNA mutation might prove significant in the detection of tumour recurrence[152] and possibly in the detection of genotoxic damage[157].

Conclusions and future prospects

It is nearly 25 years since Sanger and colleagues sequenced the human mitochondrial genome, and significant progress in the mitochondrial field continues to be made. Our understanding of the pathogenesis of mtDNA disease will greatly improve by studying the basic processes involved in replication, transcription and translation of the mitochondrial genome.

Unequivocally, mtDNA mutations are an important cause of genetic disease. The clinical variability of these disorders makes the recognition of patients with mtDNA disease a real challenge. Clinicians must be aware of its impact; accurate diagnosis requires a combination of different studies and should be carried out in specialist centres. Although the most disappointing area has been the lack of treatment for patients with mtDNA disease, several new experimental approaches are currently under investigation. It is crucial that further work and ideas are forthcoming to realistically treat or prevent the transmission of mtDNA disease to future generations. The involvement of mtDNA mutations in neurodegenerative disease, ageing and cancer is under debate. Although the development of an animal model should clarify a possible role of mtDNA mutations in ageing, further work in both human tissues and animal models is required to explain the role of mtDNA in these areas of medicine.

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Competing interests statement
The authors declare no competing financial interests.

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