

Genetic Counselling for Maternally Inherited Mitochondrial Disorders

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Abstract The aim of this review was to provide an evidence-based approach to frequently asked questions relating to the risk of transmitting a maternally inherited mitochondrial disorder (MID). We do not address disorders linked with disturbed mitochondrial DNA (mtDNA) maintenance, causing mtDNA depletion or multiple mtDNA deletions, as these are autosomally inherited. The review addresses questions regarding prognosis, recurrence risks and the strategies available to prevent disease transmission. The clinical and genetic complexity of maternally inherited MIDs represent a major challenge for patients, their relatives and health professionals. Since many of the genetic and pathophysiological aspects of MIDs remain unknown, counselling of affected patients and at-risk

family members remains difficult. MtDNA mutations are maternally transmitted or, more rarely, they are sporadic, occurring de novo (~25%). Females carrying homoplasmic mtDNA mutations will transmit the mutant species to all of their offspring, who may or may not exhibit a similar phenotype depending on modifying, secondary factors. Females carrying heteroplasmic mtDNA mutations will transmit a variable amount of mutant mtDNA to their offspring, which can result in considerable phenotypic heterogeneity among siblings. The majority of mtDNA rearrangements, such as single large-scale deletions, are sporadic, but there is a small risk of recurrence (~4%) among the offspring of affected women. The range and suitability of reproductive choices for prospective mothers is a complex area of mitochondrial medicine that needs to be managed by experienced healthcare professionals as part of a multidisciplinary team. Genetic counselling is facilitated by the identification of the underlying causative genetic defect. To provide more precise genetic counselling, further research is needed to clarify the secondary factors that account for the variable penetrance and the often marked differential expressivity of pathogenic mtDNA mutations both within and between families.

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Key Points

Genetic counselling for maternally inherited mitochondrial disorders (MIDs) is challenging due to their wide phenotypic and genotypic heterogeneity.

Reproductive choices are now available for mothers carrying pathogenic mitochondrial DNA mutations.

1 Introduction

Mitochondrial disorders (MIDs) constitute the most common group of metabolic disorders, with an estimated prevalence of 1:4200 in the population [1, 2] and diagnosed in >1 in 7500 live births [3]. Furthermore, unrecognised oligosymptomatic MIDs have a much higher prevalence than previously thought at about 1 in 400 [4]. MIDs are usually multisystem disorders, either at onset or evolving from single to multiple organ involvement as the disease progresses. The onset of disease can occur any time from the neonatal period to old age. Any combination of tissues or organs can occur, and the severity can be highly variable depending on the underlying mutation, the mutation load and disease duration [5]. MIDs are classically associated with respiratory chain defects that affect the mitochondrial electron transport chain, but it is now well-established that impairment of other mitochondrial pathways namely, β -oxidation, iron metabolism, copper trafficking, mitochondrial translation, cardiolipin remodelling (Barth syndrome), the acyl-glycerol kinase pathway (Sengers syndrome), heme synthesis, the pyruvate dehydrogenase complex (PDC deficiency) or the ketoglutarate dehydrogenase complexes may also cause MIDs [6]. MIDs can be caused by mitochondrial DNA (mtDNA) mutations (primary MIDs) or by mutations in nuclear DNA (nDNA)-located genes (nuclear MIDs). The mitochondrial genome encodes for 37 genes whereas over 1300 nuclear genes encode for critical components of the mitochondrial machinery [7]. MIDs caused by nuclear genetic defects can therefore follow an autosomal dominant (AD), autosomal recessive (AR) or X-linked pattern of inheritance. Most MIDs caused by nDNA-located genes are transmitted via an AR pattern and more rarely via an AD pattern [8]. Pathogenic mtDNA mutations are strictly maternally transmitted, but sporadic mtDNA mutations, in particular single large-scale mtDNA deletions, can also occur. Because of the complex interplay between the mitochondrial and nuclear genomes and the difficulties in predicting the transmission of heteroplasmic mtDNA mutations, genetic counselling for maternally inherited MIDs remains challenging [9]. This literature review addresses clinically relevant questions about disease penetrance, prognosis, recurrence risks and the options for preventing maternal transmission of a pathogenic mtDNA mutation to the next generation.

2 Methods

The data included in this review were identified by a literature search of MEDLINE, Current Contents, EMBASE, Web of Science, Web of Knowledge, LILACS (Literatura Latino Americana em Ciências da Saúde),

SCOPUS and Google Scholar using the search terms “maternal inheritance”, “maternally inherited” and “matrilineal”, in combination with “genetic counselling”. Randomised (blinded or open-label) clinical trials, longitudinal studies, case series and case reports were considered. Only articles published in English, French, Spanish or German between 1966 and 2017 were assessed. Appropriate papers were studied and discussed before making a decision on their suitability for inclusion in this review.

3 Results

3.1 Mitochondrial Genome

The human mitochondrial genome is a circular, double-stranded DNA molecule consisting of 16,569 base pairs (bp). It encodes for 13 proteins, which are all essential components of the mitochondrial respiratory chain complexes, two ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) [10]. Except for the D-loop, which is involved in the initiation of mtDNA replication and transcription, mtDNA is a very compact molecule with overlapping gene regions and there is little non-coding DNA [10]. mtDNA does not contain introns and the genetic code varies from the universal code. A single mitochondrion usually contains five to ten mtDNA copies. Since a single somatic cell can contain up to 1000 mitochondria, <10,000 mtDNA copies are present within a single somatic cell. MtDNA copy number in germ cells differs from that in somatic cells. In mature mammalian oocytes, mtDNA copy number goes up to 100,000–200,000 whereas sperm cells only contain about 100 mtDNA copies [11]. Overall, mtDNA represents ~1% of the total DNA content of a human cell.

3.2 Pathogenic Mitochondrial DNA (mtDNA) Mutations

Primary MIDs can arise from either mtDNA point mutations or genomic rearrangements, namely insertions, duplications, triplications or single large-scale deletions [12]. Primary mtDNA mutations may be present in all mitochondrial genomes of a cell (homoplasmy) or only in some of them (heteroplasmy). In the homoplasmic state, all of the mtDNA molecules in a cell harbour the same mtDNA sequence at a particular nucleotide position, whereas in the heteroplasmic state there is a mixture of two or more distinct types of mitochondrial genome. In the context of a pathogenic mtDNA mutation, the level of the mutant species can have an influence both on penetrance (threshold effect) and the severity of disease expression

[12]. Many MIDs only become clinically manifest when the mutant level exceeds a certain threshold, but this figure does vary widely between different mtDNA mutations [3] from as low as 8% [13] to almost homoplasmy. While the frequency of heteroplasmic mtDNA mutations has been estimated to be 1 in 200 in the general population [14], next-generation deep sequencing suggests that it is universal at low levels [15].

Nuclear genes that cause MIDs can result in mtDNA maintenance defects in the form of either multiple mtDNA deletions or mtDNA depletion. A rapidly expanding list of nuclear genes causing multiple mtDNA deletions have now been identified, including *POLG1*, *C10orf2* (*PEO1*), *TK2*, *RRM2B*, *RNASEH1* and *OPA1* [16]. Depletion is present when the amount of mtDNA is decreased by >70% of normal [17]. Mitochondrial depletion syndromes have been reported in patients carrying mutations in nuclear genes such as *POLG1*, *TK2*, *SUCLA2*, *MPV17*, *FBXL4*, *RRM2B*, *TYMP*, *PEO1*, *SUCLG1*, *DGUOK*, or *OPA1* [18]. These nuclear genes are outside the scope of this review because they cause disease with an AD or AR mode of inheritance.

3.2.1 MtDNA Point Mutations

MtDNA point mutations are maternally inherited, but de novo mutations can occur (~25%) [18], which are usually in the heteroplasmic state [19–22]. They can occur in the 22 tRNAs, the two rRNAs, one of the 13 protein-encoding genes, or in non-coding regions. The identification of a mtDNA mutation in only the proband (de novo mutation) can be due to: (1) other family members in the maternal lineage harbouring the mutation, but below the detection limit for the laboratory assay used; (2) the proband is not biologically related to other family members; or (3) the mutation has truly arisen de novo [21], with [22] or without germline mosaicism.

3.2.2 MtDNA Rearrangements

3.2.2.1 Single, large-scale mtDNA Deletions Compared with mtDNA point mutations, single large-scale mtDNA deletions usually represent de novo mutational events occurring either in an oocyte or during embryogenesis [5]. Therefore, only the proband is usually affected in the family, but there is nevertheless a low risk of transmission by the mother of an affected child. The recurrence risk for a single, large-scale mtDNA deletion has been estimated at 1 in 117 (4.1%) [20]. Single, large-scale mtDNA deletions have a wide phenotypic spectrum ranging from isolated chronic progressive external ophthalmoplegia to the more severe Kearns-Sayre Syndrome (KSS) or Pearson Syndrome (PS) [5]. Since not all large-scale mtDNA rearrangements can be detected reliably in blood samples,

muscle is the preferred tissue for long-range PCR analysis [5].

3.2.2.2 Insertions, Duplications and Triplications Pathogenic mtDNA insertions, duplications or triplications have been occasionally reported as causing MIDs, either as large-scale [23] or small-scale [24] rearrangements. In a particular case with maternally inherited diabetes and deafness (MIDD) and complex-I deficiency in fibroblasts, mtDNA sequencing revealed a heteroplasmic insertion of one or two cytosine residues in the coding region of the *MT-ND6* gene (m.14535_14536insC or CC), leading to a premature stop codon [25]. A 9-bp triplication in the *MT-CYTB* gene was detected in an African patient with isolated mitochondrial myopathy [24]. In a patient with dilated cardiomyopathy, the causative mutation was a 15 bp duplication in the *tRNA^{Pro}* gene [26].

3.2.3 Confirmation of a Mutation as Pathogenic

Because the mutation rate of mtDNA is rapid, neutral polymorphisms are common. Distinguishing between pathogenic mtDNA mutations and neutral mtDNA variants can generally be achieved using several approaches:

1. In silico computational prediction, applying programs such as CADD, CONDEL, FATHMM, MutationAssessor, PANTHER, PolyPhen-2, PROVEAN or SIFT. Computational algorithms predict the pathogenicity of a mutation based on the phylogenetic similarity among homologues (orthologues and paralogues), 3-dimensional structures, and epidemiological, clinical and genetic characteristics of the variants.
2. In vitro biochemical methods, which include confirmation of decreased mitochondrial enzyme activity in muscle, liver or fibroblasts or in an in vitro trans-mitochondrial cybrid system. The latter is an artificial eukaryotic cell line produced by introducing mitochondria from one organism into a cell of the same or different species, from which the mtDNA has been removed. A disadvantage of in vitro studies is that they cannot represent the physiological condition and cannot test as yet unrecognised activities or functions [27, 28].
3. By correlating the mutation load with the histochemical phenotype in skeletal muscle.
4. In vivo studies of knock-in transgenic animals [27].

Additionally, a mutation can be classified as pathogenic if the mutation is heteroplasmic, if the mutation is not present in healthy controls, if the type of mutation and the mutated gene are consistent with biochemical and histopathological findings, if the mutation is located in a structurally and functionally important region, or if the amino acid involved is evolutionarily conserved [29, 30].

3.3 The Mitochondrial Bottleneck

An important phenomenon during early oogenesis is the marked reduction in mtDNA copy number within the maturing cell from 200,000 copies to 200–2000 copies [31], which is referred to as the bottleneck effect [3, 5, 32]. Although the effective size of the germline mtDNA bottleneck has no precise figure, it has been estimated as between 10 and ~30–35 [31, 33]. If a mother carries a heteroplasmic mtDNA mutation, the mitochondrial bottleneck can result in significant shifts in the level of the mutant species in her population of oocytes. The mechanisms driving this bottleneck effect is under intense investigation and there is still ongoing debate whether it is an entirely random event and/or determined by the nature of the underlying mtDNA mutation [34]. Homoplasmic mtDNA mutations are not affected by the bottleneck effect.

3.3.1 Random Bottleneck Effect

The bottleneck effect is frequently defined as a rapid intergenerational switching of mtDNA types that has been largely finished by the time oocytes are mature [35]. This seems to occur during the expansion in mtDNA copy number that occurs between early and late oogenesis [33, 36–38]. The major component of the bottleneck occurs between the primordial germ cell and the primary oocyte stage [35]. The mitochondrial bottleneck effect may explain why an asymptomatic mother or a mother with a mild MID can have offspring with a highly variable phenotype ranging from normal to severe life-threatening phenotypes [5]. The bottleneck effect may also explain the intrafamilial phenotypic heterogeneity within a generation and between generations. However, the mean amount of mutant mtDNA per cell in the offspring and in their oocytes (for female children) is likely to be close to that of the mother [35, 39, 40].

3.3.2 Conditional Bottleneck Effect

There is preliminary evidence that the shift in heteroplasmy levels during oogenesis may not only be due to a random drift process, but could also be influenced by the specific nature of a particular mtDNA variant, implying that the mitochondrial bottleneck could have selective properties [14, 39]. In one study of 577 mother–child pairs transmitting the m.11778G > A, m.3460G > A, m.8344A > G, m.8993T > G/C and m.3243A > G mtDNA mutations, there was no evidence for selection during transmission, but in another study there appeared to be selection against novel heteroplasmies, which are likely to be deleterious [41]. Different mutations segregated at different rates in human pedigrees [14]. Patients harbouring the

m.8993T > G/C mutation showed more rapid segregation of heteroplasmy than the m.11778G > A, m.8344A > G or m.3243A > G mutations [14]. This trend is consistent with a tighter mitochondrial bottleneck in m.8993T > G/C pedigrees, suggesting that there are different bottlenecks depending on the underlying mtDNA mutation [14]. It is therefore conceivable that non-synonymous, single-base pair substitutions in the background mtDNA haplotype could also result in geographic, ethnic or inter-familial differences of segregation patterns [12]. To make the story more complex, genotypic shifts probably occur post-zygotic [42, 43] as has been shown in mice [42]. These intriguing findings need to be explored further to better understand the different inheritance patterns observed in human pedigrees transmitting pathogenic mtDNA mutations [14].

3.4 Mitochondrial Heteroplasmy

Heteroplasmy levels can vary considerably, not only within a generation or between generations but also between tissues and organs of the same individual. Conceptually, higher levels of the mutant mtDNA species should result in a more severe phenotype, but there is not always a straightforward correlation between mutant load and disease severity [44]. For example, a 48-year-old woman with diabetes mellitus, hearing loss and retinal dystrophy carried the m.3243A > G mutation at heteroplasmy rates of 8% in blood, 6% in hair follicles and 16% in buccal mucosa cells, whereas her asymptomatic 28-year-old son carried the mutation at higher levels of 23, 15 and 16%, respectively [30]. An important conclusion to note from this is that the amount of heteroplasmy in blood or unaffected tissues does not always reflect the mutant load in affected post-mitotic tissues [30]. The mutant load is almost always lower in blood than in muscle and for the m.3243A > G mutation there is good evidence that it falls with time [45]. The load of mutant mtDNA appears to be relatively uniform at birth [46], but often falls in blood [44] and increases in post-mitotic tissues such as brain and muscle [9, 47]. The mechanisms underlying these changes are not well-understood but likely depend both on differences between wild-type and mutant mtDNA in the rate of mtDNA synthesis [48] and mtDNA turnover. Decrease of the m.3243A > G mutant load in blood could be also due to death of highly mutated stem cells in the hematopoietic tissue [49]. This makes estimating mutant loads difficult, as levels of mutant mtDNA in blood, which is accessible for sampling, may be a poor reflection of the mutant load in tissues causing symptoms. In other patients with MIDs, however, the level of heteroplasmy correlates more closely, but not invariably, with the phenotype, such as in carriers of the m.8993T > G mutation [30]. Depending on the mutant load, a carrier of

this mutation will develop a severe neurodegenerative Leigh-like syndrome (>90%), the neuropathy, ataxia, and retinitis pigmentosa (NARP) phenotype characterised by neurogenic weakness, ataxia and retinitis pigmentosa (60–75%), or remain asymptomatic (<60%) [30]. The mutant load for these groupings are correspondingly higher for the milder m.8993T > C mutation [50].

As a result of the mitochondrial bottleneck, the degree of heteroplasmy varies considerably in the oocyte population of a female carrier harbouring a heteroplasmic mtDNA mutation [51]. Prenatal testing, in which mtDNA is sampled through amniocentesis or chorionic villus sampling (CVS) therefore carries the risk that it may not accurately reflect the mutation load in the child's tissues, especially those most at risk of disease [52]. Nevertheless, it definitely reduces the risk of severe disease and some couples find this acceptable [45]. Phenotypic heterogeneity can be additionally explained by the involvement of disease-modifying nuclear genes [30].

3.5 Variable Penetrance

Some mtDNA mutations exist mostly in the homoplasmic state although, despite this, they can exhibit variable penetrance. A classical example is Leber hereditary optic neuropathy (LHON), which is the most common primary MID in the population with an estimated minimum prevalence of about 1 in 30,000 [53]. LHON is characterised by bilateral subacute visual loss with a peak age of onset in the second or third decades of life. About 90% of cases are due to one of three point mtDNA mutations (m.3460G > A in *MT-ND1*, m.11778G > A in *MT-ND4* or m.14484T > C in *MT-ND6*), which all affect critical complex-I subunits, and the majority of these mutations are detected at homoplasmic levels. The paradox is that only ~50% of male carriers and ~10% of female carriers will lose vision in their lifetime due to the influence of secondary genetic, hormonal or environmental modifiers [54, 55]. Although the majority of those who will lose vision will do so before the age of 50 years and the risk is significantly lower for female carriers, it is not currently possible to accurately predict who is going to lose vision and when. MtDNA copy number appears to influence penetrance, given the observation that symptomatic individuals have less mtDNA per cell than do asymptomatic carriers of the same mutation [55]. This could be due to different rates of mitochondrial biogenesis [55] and/or turnover [56].

3.6 Tissue Specificity

MIDs frequently present with non-specific multisystem manifestations and, as a result, their diagnosis and proper

management is often delayed [5]. To compound the diagnostic challenges, patients can present at any age, with almost any affected body system and with a wide range of clinical severity [5]. The phenotype may also differ between paediatric and adult cases. In paediatric cases, encephalopathy, muscle hypotonia, lactic acidosis, epilepsy, cardiomyopathy and psychomotor delay are the most frequently reported features [57]. In adult-onset cases, the predominant phenotypic features include sensorineural deafness, diabetes, myopathy (ptosis, ophthalmoplegia, limb weakness, respiratory insufficiency), peripheral neuropathy, visual failure (cataract, optic neuropathy, pigmentary retinopathy), cardiac, gastrointestinal, renal, other endocrine or central nervous system involvement (ataxia, movement disorders, seizures, cognitive impairment). The heterogeneous tissue specificity and variable expressivity that can be associated with the same pathogenic mtDNA mutation contribute to the challenging nature of genetic counselling for this group of disorders with regards to prevention, recurrence risk and prognosis.

3.7 Genetic Counselling

Genetic counselling is the process of providing individuals and families with information on the nature, inheritance and implications of genetic disorders to help them make informed medical and personal decisions [58]. A detailed family history and genetic testing to clarify the genetic status of both clinically affected and unaffected family members are essential in guiding the discussion. Up to 80% of the childhood-onset MIDs are due to mutations in nDNA genes whereas 75% of adult-onset MIDs are due to mtDNA mutations [59]. Genetic counselling will therefore differ in these two categories of patients and other considerations will take different levels of importance, such as educational needs, occupational rehabilitation or reproductive options.

3.7.1 Prerequisites for Genetic Counselling

From a management and prevention standpoint, appropriate genetic investigations in a patient with suspected MID is essential [5]. A confirmed molecular diagnosis will help to better understand the natural history of the disease, the recurrence risk to other family members and the option of familial contact tracing [5]. With regard to genetic counselling, it is additionally important to know if a given mtDNA mutation is homoplasmic or heteroplasmic [60]. A homoplasmic mtDNA mutation will be transmitted to all offspring, but disease penetrance can vary as in LHON [61]. For a heteroplasmic mtDNA mutation, the level can vary markedly in the same sibship as a result of the mitochondrial bottleneck, which can influence the pattern and severity of disease expression.

3.7.2 Objectives of Genetic Counselling

Genetic counselling aims to provide accurate information about the prognosis (prediction) of affected individuals, the recurrence risk for other family members and reproductive choices (prevention) [10]. Genetic counselling for patients carrying mtDNA mutations is determined by the specific mutation involved, whether the mutation is heteroplasmic or homoplasmic, by disease penetrance and by the associated phenotypic spectrum [5]. Genetic counsellors are trained health professionals that work in close collaboration with physicians and clinical scientists as part of a multidisciplinary team. Such a concerted approach is needed to develop the best management approach based on the individual needs and expectations of patients and their families, with the aim of helping them to build effective coping strategies [62].

3.7.3 Prediction of Phenotype and Progression

It is very difficult to predict the clinical progression associated with most pathogenic mtDNA mutations. Caution should also be exercised when mutation levels have been quantified in non-affected tissues, such as blood, as the mutant load might be significantly higher in the tissues most at risk of disease. Even for homoplasmic mtDNA mutations, there can be differential vulnerability to mitochondrial dysfunction in different tissues or organs [30]. LHON, for example is mostly caused by homoplasmic mtDNA mutations, but this MID is characterised by both marked incomplete penetrance and a male bias [63]. The m.14484T > C mutation carries the best visual prognosis, but it is not possible to predict which patients will experience spontaneous visual recovery, nor the timing or the extent [64].

3.7.4 Recurrence Risk

3.7.4.1 Parents of a Proband The father of a proband carrying an mtDNA mutation will not be a carrier due to the strict maternal inheritance of mtDNA [58]. The mother of a proband will invariably carry the pathogenic mtDNA mutation, except in rarer cases of a de novo mutation, and she may or may not be clinically affected (Table 1) [58].

3.7.4.2 Offspring of a Proband MtDNA Point Mutations A male patient carrying an mtDNA point mutation can be reassured that his children are not at risk of inheriting his genetic defect [63]. A female carrier of a homoplasmic mtDNA mutation will transmit her pathogenic mtDNA variant to all of her children. If the mutation is heteroplasmic, the level that is transmitted to each child can fluctuate considerably due to the bottleneck effect [63] but reflecting the

mother's load of mutant mtDNA. For instance, ~30% of children born to mothers harbouring 5–25% of m.3243A>G in urinary epithelial cells will have <5% and in many undetectable m.3243A>G [65]. For mothers harbouring >25% m.3243A>G, mutant will be detectable in almost all offspring [65], though the published figures probably over-estimate the risks due to ascertainment bias. Since the level of the mutation in amniocytes or chorionic villi may differ from the mutation load in other cell populations, prenatal testing for females carrying a heteroplasmic mtDNA mutation poses significant challenges [63]. In a study of 33 children and 72 adults carrying an mtDNA point mutation, a de novo frequency of 24.6% was reported [19]. Prenatal diagnosis (PND) for the mothers of four patients with de novo mtDNA mutations revealed the absence of the mutant mtDNA species in all 11 oocytes/embryos tested [19]. Based on a literature survey, 137 patients harbouring de novo mtDNA mutations have been previously reported and PND was performed for the mothers of nine probands [19]. Recurrence occurred in only one of these nine mothers in two of her subsequent pregnancies, presumably due to germline mosaicism [19]. The overall recurrence risk of de novo mtDNA point mutations to the same parents therefore seems to be relatively low [19].

MtDNA Rearrangements For genetic counselling of a patient carrying a single mtDNA deletion, it is essential to assess if the deletion is de novo or, more rarely, maternally inherited [30]. In the rare cases with maternally inherited major rearrangements [67], i.e. tandem duplications and/or deletion dimers are almost always present in addition to the single deletions in skeletal muscle [67]. The most parsimonious explanation is that the first mutant molecule was a tandem duplication (likely occurring during replication) and that related molecules were generated by homologous recombination [68]. If a single mtDNA deletion is de novo, having arisen sporadically during embryonic development, the probability of transmitting it to the next generation is extremely low, except in cases of mosaicism of the germline oocytes [12]. A clinically unaffected mother is highly unlikely to have more than one affected child. However, a clinically affected mother has about a 1 in 24 risk of having a clinically affected child, which can be associated with the presence of a partially duplicated mtDNA in the mother [69].

3.7.4.3 Siblings of a Proband The risk to siblings of an affected proband depends on the genetic and clinical status of the mother. If the mother carries an mtDNA point mutation, all siblings of a proband are at risk of inheriting the pathogenic variant, although the level can vary depending on whether the mutation is heteroplasmic or homoplasmic [58]. If the proband harbours a single mtDNA deletion, the recurrence risk to a sibling is 4% if the mother is clinically affected [69].

Table 1 Estimated risks of a female carrier having a child with symptomatic mitochondrial disease based on the heteroplasmic/homoplasmic state of the mtDNA mutation

MtDNA mutation	Level of the mutation	Risk (%)	Likely phenotype	PND	References
Point mutation					
m.3243A > G	Heteroplasmy	np	MIDD or MELAS	Yes ^a	[65]
m.3460G > A	Heteroplasmy	np	LHON	Yes	None
m.8344A > G	Heteroplasmy	np	MERRF	Yes	None
m.8993T > G/C	Heteroplasmy	np	Leigh/NARP	Yes	[50]
m.11778G > A	Homoplasmy	50 ^b	LHON	No	[53–55]
m.11778G > A	Homoplasmy	10 ^c	LHON	No	[53–55]
m.13513G > A	Homoplasmy	np	Leigh	Yes	None
m.14453G > A	Heteroplasmy	np	MELAS	Yes	None
m.14484T > C	Heteroplasmy	np	LHON	Yes	[70]
Rearrangement					
m.8470_13446del14977	Heteroplasmy	4.1	KSS, PS, CPEO	Yes ^a	[20]
m.14535_14536insC or CC	Heteroplasmy	Low ^d	MIDM	Yes ^a	[25]
m.14535_14536insC or CC	Heteroplasmy	4 ^e	MIDM	Yes ^a	[25]

np not provided, PND prenatal diagnosis

^a With the caveat discussed in the text

^b Son

^c Daughter

^d Asymptomatic female carrier

^e Symptomatic female carrier

3.8 Preventing Maternal Transmission of mtDNA Mutations

3.8.1 Oocyte Donation

Oocyte donation is a low-risk strategy to eliminate the transmission of a pathogenic mtDNA mutation from mother to child. The donated oocyte is fertilised with the partner's sperm and the resulting embryo is placed in the uterus of the carrier mother who then becomes the biological, but not the genetic mother. Potential issues relating to genetic versus biological parentage should be put in perspective. As >10% of the UK population do not identify their genetic fathers correctly [71], a genetic link does not appear to be a prerequisite for good intrafamilial relationships. Overall, oocyte donation is not a common reproductive choice for women of childbearing age who are known to harbour a pathogenic mtDNA mutation.

3.8.2 Prenatal Diagnosis

Prenatal testing and prenatal counselling after conception is only possible if the causative pathogenic mutation is known [3]. A mother who carries a homoplasmic mtDNA mutation should not be offered CVS or an amniocentesis to

evaluate the risk of disease for the fetus, which will also be homoplasmic. This is because it will not help assess the risk of recurrence (see Sect. 3.5 for discussion of unknown factors affecting penetrance). Interpretation of PND testing in mothers who carry a heteroplasmic mtDNA mutation is complex since the mutational load in fetal tissues tested (amniocytes or chorionic vili) may differ from the eventual level in the tissues that are most at risk of a particular mtDNA mutation [58]. Nevertheless, most studies show a positive correlation between mutant loads in fetal tissue and amniocytes [42, 72].

In the case of heteroplasmic mutations, interpretation of PND depends on factors such as the heteroplasmy rate, threshold level, phenotypic expression of the mutation in maternal relatives and the strength of the genotype/phenotype correlation, which should be considered and carefully discussed with the mother [3]. Counselling is particularly difficult if fetuses harbour mtDNA mutations with intermediate heteroplasmy and unclear genotype/phenotype correlation [3]. The most common mtDNA mutation studied for PND is the mutation m.8993T > G [30]. In a study of 62 prenatal samples (17 mtDNA and 45 nDNA), it was observed that the analysis of heteroplasmy levels in other family members can be helpful in interpreting the prenatal mtDNA test result, particularly in cases

of rare mtDNA mutations or when intermediate heteroplasmy rates (30–70%) have been detected [3]. After PND and termination of pregnancy, at least 11 cases of MID were prevented, three of which were related to mtDNA mutations (m.3243A > G, m.14453G > A, m.13513G > A) [3].

3.8.3 Preimplantation Genetic Diagnosis

Although preimplantation genetic diagnosis (PGD) remains controversial, it is a potential option that could be applied to lower the recurrence risk. This approach has raised a number of concerns [73], in particular that the mutant load cannot be accurately predicted in all tissues of the resulting embryo [5]. This concern is because of a single case where the child's mutant load exceeded that of the blastocyst biopsy [74]. However, in this instance, the embryo was not biopsied until the blastocyst stage. Previous studies have shown that the variable tissue segregation of mtDNA variants that occurs in later stages of embryonic development is not yet present in the preimplantation cleavage-stage embryo, in which heteroplasmic mtDNA tends to be uniformly distributed in all blastomeres [32, 39]. Thus, accurate assessment of the embryo's mutant load may be crucially dependent upon a biopsy procedure being done <4 days after fertilisation, just before the blastocyst forms [75]. Hence, if the zygote is sampled at an earlier stage of development, the mutant load can be more accurately assessed. Nevertheless, in women where only a small number of embryos are generated it can be ethically challenging to decide which embryos should be implanted to maximise the chance of having an unaffected child. PGD is not an option for mothers who carry homoplasmic mtDNA mutations.

3.8.4 Prevention of Germline Transmission

Mitochondrial replacement therapy (MRT), which involves a modification of conventional in vitro fertilization (IVF) techniques, has been proposed as a possible reproductive strategy [76]. The two techniques of mitochondrial donation being optimised are pronuclear transfer and metaphase-II spindle transfer. In pronuclear transfer, the mother's oocyte is first fertilised with the father's sperm, followed by the removal of the parental pronuclei. These are then transferred into an enucleated mitochondrial donor zygote at the same stage, harbouring only wild-type mtDNA [77, 78]. In metaphase-II spindle transfer, the mother's metaphase-II spindle, which contains the maternal nDNA, is transferred into an enucleated mitochondrial donor oocyte and then fertilised by intracytoplasmic sperm injection [79, 80]. Both IVF techniques have shown promising results with minimal carryover of mutant

mtDNA, but a number of safety concerns have been raised, magnified by the fact that the germline is being altered [81, 82]. While the technique is likely to reduce the load of mutant mtDNA, segregation may be unpredictable, resulting in reversion towards the pathogenic mtDNA type [78, 83]. The cause of this segregation is not clear, though one group identified a polymorphism that may drive a replicative advantage [83]. Two strategies have been suggested for countering this phenomenon. Firstly, segregation may be reduced by matching the mtDNA haplotype of the donor and recipient [84, 85]. Secondly, it might be possible to recruit the cell's own quality control mechanisms to target the pathogenic mutant mtDNAs [86]. There remains the possibility of significant metabolic problems arising from a mismatch [87, 88].

There are also important legal and ethical issues that need to be carefully addressed before considering the wider clinical implementation of mitochondrial donation for prospective mothers harbouring homoplasmic mtDNA mutations [63, 89]. Concerns have been raised as reversal to the mutant cell line may occur in some MRT children, possibly due to polymorphisms in the CSB-II region of the D-loop [83]. Certain mtDNA haplotypes may confer embryonic stem cells with faster growth and proliferative advantages [83]. Despite these potential risks, the UK Parliament voted in favour of changing the law in February 2015 to allow mitochondrial donation to be offered as a reproductive option if the technique can be shown to be effective with the benefits outweighing the potential risks [90]. In the first reported case of mitochondrial donation, which was carried out in Mexico in 2016, the child was reported to be healthy at 7 months of age with a mutational load of 2.36–9.23% in his tested tissues. The boy's mother is heteroplasmic for the m.8993T>G mtDNA mutation in MT-ATP6, which in addition to NARP can cause Leigh syndrome when present at high mutational levels. Whilst this is encouraging, the effectiveness of reducing the mutant load remains unclear as the mutant load in the oocyte was not reported [91]. Furthermore, long term follow up is needed as mtDNA segregation could occur later in the child's life.

3.9 The Patient and Family Perspective

The diagnostic process is often perceived as complex by patients and their families, and it is frequently accompanied by strong emotions that are partly influenced by the patient's support system and the approach of the healthcare professionals overseeing their care [5]. Due to the uncertainty about the diagnosis, psychological distress may arise, including feelings of blame and guilt, which can precipitate maladaptive coping mechanisms [5]. Based on a

study of 14 parents searching for a diagnosis for their children, their perception of the diagnostic process was accompanied by both emotional and sociological responses [92]. Their emotional experience meant coming to terms with the health status of their affected child, whereas their sociological experience reflected their interactions as parents with members of the healthcare system and their access to support systems [92]. Frustration was a major reaction as they encountered obstacles when searching for their child's diagnosis [92]. Only a few studies have explored the psychological consequences to patients and their relatives following the diagnosis of a MID and further work is needed regarding how to best support these individuals in coping with these emotionally charged and difficult personal life events [5].

4 Conclusions

Genetic counselling for MIDs remains an imperfect science due to the genetic complexity of this group of disorders and our incomplete understanding of the complex factors that drive tissue specificity and disease expressivity. A multi-disciplinary and holistic approach is crucial to better understand how patients and their relatives deal with the anxiety and fears brought by an unexpected genetic diagnosis. Genetic counsellors have an important role to play in effectively communicating the information provided by a genetic test, and in making sure that it is properly processed to empower patients and their relatives in making the best decisions based on their own personal circumstances. A number of reproductive choices are now available for prospective mothers carrying pathogenic mtDNA mutations with the advent of mitochondrial donation and other potential germline strategies emerging. More than ever, the proper framework needs to be put in place to allow for careful regulation and to encourage an informed societal debate about the risks and benefits of these experimental therapies.

Compliance with Ethical Standards

Conflicts of interest JP, JF and PYWM declare no conflicts of interest.

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