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Review

Mitochondrial encephalomyopathies: the enigma of genotype versus phenotype

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Abstract

Over the past decade a large body of evidence has accumulated implicating defects of human mitochondrial DNA in the pathogenesis of a group of disorders known collectively as the mitochondrial encephalomyopathies. Although impaired oxidative phosphorylation is likely to represent the final common pathway leading to cellular dysfunction in these diseases, fundamental issues still remain elusive. Perhaps the most challenging of these is to understand the mechanisms which underlie the complex relationship between genotype and phenotype. Here we examine this relationship and discuss some of the factors which are likely to be involved.

Keyword: Mitochondrial encephalomyopathy

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1. Introduction

The mitochondrial encephalomyopathies are a heterogeneous group of diseases which are mostly caused by inborn errors of oxidative phosphorylation (reviewed in [1–4]). Despite the wide range of clinical presentations, a number of distinctive syndromes have been identified. The most notable of these are: (1) progressive external ophthalmoplegia (PEO) and the Kearns-Sayre syndrome (KSS), (2) mitochondrial encephalomyopathy, lactic acidosis and strokes (MELAS), (3) myoclonic epilepsy with ragged-red muscle fibres (MERRF), (4) mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), (5) Leigh's syndrome, and (6) the syndrome of neurogenic weakness, ataxia and retinitis pigmentosa (NARP). In addition to distinctive encephalomyopathies, mutations in oxidative phosphorylation genes have been identified in Leber's hereditary optic neuropathy (LHON), Pearson's bone marrow-pancreas syndrome, diabetes mellitus and deafness, hypertrophic and dilated cardiomyopathy, multiple symmetrical lipomatosis, non-syndromic and aminoglycoside induced sensorineural deafness, fatal infantile hepatopathy, recurrent myoglobinuria and other relatively pure myopathies as well as in various encephalomyopathies and multisystem diseases which lack distinctive features and cannot be assigned to any of the above categories. Many of these disorders are maternally inherited but some are sporadic and others appear to be transmitted as mendelian traits. Ragged-red muscle fibres (RRF), an established morphological hallmark of these disorders, are uncommon in Leigh's syndrome and in LHON and have not yet been reported in NARP.

With the exception of defects in Complex II [5], most of the well characterised inborn errors of oxidative phosphorylation have been linked to mutations in the mitochondrial genome (mtDNA), a highly compact, double stranded circular molecule of 16 569 nucleotide pairs which codes for 13 of the 90 or so polypeptide components of this major energy generating pathway assembled in the mitochondrial inner membrane [1–4,6,7]. Various pathogenic mtDNA abnormalities have been identified, the commonest being large-scale rearrangements (single deletions, multiple deletions, duplications) and point mutations in transfer RNA (tRNA), or protein encoding genes. Virtually all these disorders are characterised by mtDNA heteroplasmy in that mutated and wild-type genomes co-exist in variable proportions and with tissue-to-tissue and cell-to-cell differences within the same individual. Whether mtDNA heteroplasmy extends to the individual mitochondrion is not known.

Although various cell culture systems have been used to study the functional consequences of some of the more common mtDNA mutations [8–16], their precise roles in disease pathogenesis and the mechanisms which account for observed disparities between mtDNA genotype and clinical phenotype have not been resolved. The association of different syndromes with the same mutation [17–23] and different mutations with the same syndrome [24–31] has prompted speculation that additional mechanisms such as the genetic background [32–35], immunological [36] or environmental factors [37], or even the ageing process itself [38] maybe important contributory determinants of phenotypic expression.

Here we review the range of disease phenotypes associated with some of the more common mtDNA mutations and discuss possible mechanisms which might account for such remarkable clinical diversity.

2. MtDNA mutations involving protein encoding genes

For reasons which remain unclear, some mtDNA mutations appear to be relatively specific in their clinical manifestations, whereas the majority are not (Tables 1–5). The former situation currently applies to the primary LHON mutations [38,39] and to the T8993G transversion in the ATPase 6 subunit gene originally identified in NARP [40]. Studies of additional pedigrees have shown that when the proportion of the NARP 8993 mutation in blood or muscle exceeds 90% it causes Leigh's syndrome [41–43]. Individuals harbouring mutant levels of 70–90% either present with NARP or with varying degrees of mental subnormality and developmental delay [43,44]. Levels below 70% are usually asymptomatic. Atypical features appear to be uncommon, but stoke-like episodes suggestive of the MELAS phenotype and PEO have been described [43,45] and hypertrophic cardiomyopathy was a novel clin-

ical manifestation in one infant with Leigh's syndrome associated with the T8993G mutation [46].

The T8993G mutation changes a highly conserved leucine for arginine in the ATPase 6 subunit which is a component of the proton channel of Complex V. Studies of cell culture systems virtually homoplasmic for the NARP mutation have shown ~50% reduction in State III respiration and in the ADP/O ratio [14] and a similar decrease in the rate of synthesis of ATP [47].

Four other pathogenic mutations in ATPase 6 have been identified (Table 1). One of these, a T8993C transition, has also been associated with Leigh's syndrome [31,48]: two further T-to-C transitions at 8851 and 9176 were originally associated with bilateral striatal necrosis [49,50] and a fourth at 9101 with LHON [51]. More recently, however, the T9176C transition has been described in Leigh's syndrome [52,53] and in sudden infant death [53]. None of the ATPase 6 mutations identified to date has induced significant mitochondrial proliferation (RRF) in skeletal muscle, but at an ultrastructural level abnormal mitochondria with paracrystalline inclusions have been observed [45].

The three primary LHON mutations are each associated with a highly tissue-specific phenotype which causes subacute and severe visual loss especially in young adult males [38,39]. The G11778A transition is by far the commonest and accounts for over 50% of all cases world-wide. The clinical features associated with the three primary LHON mutations are similar except that the chances of some visual recovery appear to be greater with the 14484 mutation than with the other two [39]. The predominance of affected males and the observation that some males and the majority of females virtually homoplasmic for one of the LHON mutations never develop visual loss suggests that the mutation itself is not the sole determinant of the disease phenotype. A putative visual-loss susceptibility locus on the X chromosome has been invoked to explain the male bias [34] and the occurrence of a multiple sclerosis

Table 1
MtDNA mutations in protein-encoding genes (maternal or sporadic)

Gene	Mutation	Phenotype	Ref.
ND1	T3308C	MELAS	[27]
	G3460A	LHON ± MS	[38,39]
ND4	A11696G	LHON and/or dystonia	[55]
	G11778A	LHON ± MS	[38,39]
ND5	G13513A	MELAS	[26]
ND6	G14459A	LHON and/or dystonia	[54]
	T14484C	LHON	[38,39]
	T14596A	LHON and/or dystonia	[55]
Cytochrome *b*	G15615A	Exercise intolerance	[208]
	(+D-loop duplication)		[209]
COX I	5-bp deletion	Motor neuron-like disease	[58]
COX III	G9952A	Recurrent encephalopathy	[57]
	T9957C	MELAS	[25]
	15-bp deletion(9486–9502)	Cramps and myoglobinuria	[56]
ATPase 6	T8851C	Striatal necrosis	[49]
	T8993G	NARP	[40,41,43,45]
		NARP and stroke	[43,45]
		NARP and PEO	[45]
		Cerebral palsy	[44]
		Leigh's syndrome	[41–43,45]
		Leigh and cardiomyopathy	[46]
	T8993C	Leigh's syndrome	[48]
	T9101C	LHON	[51]
	T9176C	Striatal necrosis	[50]
		Leigh's syndrome	[52,53]
		Sudden infant death	[53]

(MS)-like illness in some affected females with the 11 778 or 3460 mutations has prompted speculation that autoimmune mechanism may contribute to disease pathogenesis [36,39].

In contrast to the primary LHON mutations, allelic mutations in ND1, ND4 and ND6 have been associated with somewhat different clinical phenotypes (Table 1). The G14459A transition in ND6 and the A11696G transition in ND4 have been associated with LHON and/or dystonia [54,55] and the T3308C in ND1 with MELAS [27]. Another point mutation in a Complex I gene, a G13513A transition in ND5 has also been associated with the MELAS phenotype [26].

Similar phenotypic heterogeneity has been observed with allelic mutations in the cytochrome oxidase subunit III (COX III) gene. Of the three mtDNA abnormalities which have been described (Table 1), one a T9957C mutation was associated with MELAS [25] and the other a 5-bp in-frame microdeletion with muscle cramps and myoglobinuria [56]. The third COX III mutation which introduced a premature stop codon 13 amino acids upstream from the C-terminus, was associated with a unique clinical phenotype characterised by recurrent episodes of coma with virtually complete recovery between attacks [57]. Unlike the other two COX III mutations, the muscle biopsy on this patient lacked RRF but the majority of the muscle fibres showed markedly reduced COX activity when examined histochemically, a finding which was confirmed on biochemical analysis [57]. Recently, a heteroplasmic 5-bp out-of-frame deletion in the COX I subunit gene has been identified in an adult male with a motor neurone disease-like syndrome, characterised by a progressive spastic quadraparesis, speech and swallowing difficulties and minor lower motor neurone signs [58]. Muscle histochemistry showed RRF together with a high proportion of COX deficient fibres and an isolated COX deficiency was confirmed on biochemical analysis. Immunoblot analysis and immunostaining of muscle in each of the two patients with microdeletions in COX genes showed decreased immunoreactivity for several COX subunits suggesting impaired assembly or decreased stability of the holoenzyme [56,58].

Table 2
MtDNA mutations on the $tRNA^{Lys}$ gene (maternal or sporadic)

Mutation	Phenotype	Ref.
G8313A	Gastrointestinal dysfunction, seizures, myoclonus, dementia, ataxia, deafness, pigmentary retinopathy, axonal neuropathy	[72]
A8344G	MERRF	[59–61]
	MERRF-MELAS overlap	[60–62]
	Encephalomyopathy without myoclonus	[23,60,61]
	PEO, myopathy	[23,61]
	Myopathy, cardiomyopathy	[23]
	Myopathy alone	[23]
	Leigh's syndrome	[31,63]
	Multiple symmetrical lipomatosis	[22]
T8356C	MERRF	[30]
	MERRF-MELAS overlap	[68,69]
G8363A	Cardiomyopathy, deafness, ataxia, ophthalmoparesis	[71]
	MERRF	[70]

3. MtDNA mutations involving tRNA genes

The relatively specific manifestations associated with the LHON and NARP mutations contrast to some extent with the phenotypic heterogeneity associated with the common MERRF (myoclonic epilepsy with RRF) mutation, an A8344G transition in the mitochondrial $tRNA^{Lys}$ gene (Table 2). Most index cases present with myoclonus and ataxia, the core clinical features of MERRF, and like NARP patients they have quite high levels of the mutation in blood ($>30\%$); unlike the NARP mutation, however, levels in muscle and other tissues are usually much higher ($>70\%$) [59–61]. Atypical phenotypes are not uncommon and include PEO, encephalomyopathy without myoclonus, encephalomyopathy with recurrent strokes, multiple symmetrical lipomatosis, myopathy plus cardiomyopathy or myopathy alone [22,23,61,62]. Like the NARP mutation, the A8344G transition has also been associated with Leigh's syndrome [31,63]. Although RRF are invariable in MERRF they are absent in some atypical cases including one patient with Leigh's syndrome, whose muscle was virtually homoplasmic for the mutation [63].

Studies of the effects of the 8344 mutation in cultured myotubes [11] and in cytoplasmic hybrids [8,64], formed by fusing patient derived cytoplasts

(enucleated cells) to human cell lines devoid of mtDNA (ρ^0 cells), have shown that as little as 10% wild-type mtDNA is sufficient to prevent expression of the mutant phenotype. Below this level there is a rapid decline in respiratory chain activity and in the synthesis of mtDNA encoded proteins, especially those with a high lysine content. In cultured myoblasts, however, the translation defect occurs with lower levels of the mutation [11,15] and in one study appeared to preferentially affect the synthesis of COX subunits [15]. Expression of the phenotype with lower levels of the MERRF 8344 mutation has also been observed in platelet derived cytoplasmic hybrids [65]. The mechanism of these differences is not known, but could reflect the much lower copy number of mtDNA molecules per organelle in myoblast and platelet mitochondria. Proteins with altered electrophoretic mobilities have been observed in cultured myotubes and cybrids harbouring the 8344 mutation, but the effects of these abnormal translation products on respiratory chain function and assembly have not been studied. They are thought to arise from premature termination of translation at or near lysine codons due to defective aminoacylation of the mutant MERRF $tRNA^{Lys}$ by its cognate lysyl-tRNA synthetase [66]. If 10% wild-type mtDNA is sufficient to rescue myotubes and cybrids, however, it seems likely that even lower levels may be sufficient in vivo. A notable feature of the common MERRF mutation is its presence in universally high levels even in organs which appear to function normally during life and show no histological abnormality [23,63]. A similar discrepancy between the proportions of mutant mtDNA and neuronal cell loss has been observed in different regions of autopsied MERRF 8344 brain [67].

Three other pathogenic mutations in the $tRNA^{Lys}$ gene have been described (Table 2). A T-to-C transition at 8356 has been associated with MERRF [30] and with the MERRF-MELAS overlap [68,69]. A G-to-A transition at 8363 has also been identified in MERRF [70] and in two unrelated families with a complex multisystem disease characterised by hypertrophic cardiomyopathy, sensorineural deafness, ataxia and PEO without myoclonus [71]. More recently a novel G8313A mutation in the $tRNA^{Lys}$ gene has been identified in a young boy who presented at the age of four with severe gastrointestinal dysfunction and pseudo obstruction closely resembling the MNGIE phenotype [72]. He subsequently went on to develop myoclonus, seizures, progressive mental regression, cerebellar ataxia, pigmentary retinopathy, deafness and a peripheral axonal neuropathy and died in his mid teens. In vitro studies have shown that both the 8344 and the 8356 $tRNA^{Lys}$ mutations have similar functional consequences and both lead to the synthesis of abnormal translation products [64,65,73].

Perhaps the most complex of all the known mtDNA defects is the common MELAS mutation, an A3243G transition in the mitochondrial $tRNA^{Leu(UUR)}$ gene (Table 3). This single base change in a mitochondrial gene involved in transcriptional control as well as in RNA processing and translation [74] accounts for over 80% of patients with MELAS [18,75,76] but it also occurs in sporadic and maternally inherited syndromes with PEO, sometimes closely resembling the KSS phenotype [18,19,75,77,78], and in diabetes mellitus and deafness [20,79], encephalomyopathies without strokes [18,19,77] and myopathy alone [18,77], or associated with recurrent episodes of respiratory failure [21]. In a large group of unselected patients harbouring the MELAS 3243 mutation, the majority did not have the MELAS phenotype [18]. As with the NARP mutation, there appears to be some correlation between clinical phenotype and the percentage of mutant mtDNA in muscle which tends to be higher in early onset cases with typical MELAS than in late onset MELAS or in patients with other clinical phenotypes [18,19,77,78].

The precise mechanism of the A3243G transition in disease pathogenesis remains unknown. In addition to possibly altering the structure and function of one of the leucyl tRNAs, the mutation is located within a tridecamer sequence which binds the transcription termination factor mTERF [80]. The binding of mTERF at this site normally serves to selectively increase the rate of transcription of the upstream 12S and 16S rRNA genes (but not the downstream heavy-strand genes), thereby ensuring that there are sufficient 12S and 16S rRNAs for the translation of mRNAs encoding all 13 polypeptides. Although the binding of mTERF to the MELAS template was found to be greatly reduced in vitro [80], there is no evidence that transcription

Table 3
MtDNA mutations in the $tRNA^{Leu(UUR)}$ gene (maternal or sporadic)

Mutation	Phenotype	Ref.
A3243G	MELAS	[17,18,75,76,78]
	MERRF-MELAS overlap	[18,19,75,77]
	PEO ± retinopathy and/or deafness	[17–19,75,77,78]
	KSS	[19,78]
	Encephalomyopathy without stroke	[18,19,77]
	Diabetes ± deafness	[20,79]
	Myopathy	[18,77]
	Myopathy with respiratory failure	[21]
A3243T	Encephalomyopathy	[90]
T3250C	Myopathy with respiratory failure	[91]
A3251G	PEO, myopathy, psychiatric disordersudden death	[92]
A3252G	Dementia, retinopathy, diabetes, hypoparathyroidism, paraparesis, heart block, renal failure	[93]
C3254G	Myopathy, cardiomyopathy	[94]
C3256T	PEO, retinopathy, deafness, seizures, myoclonus, hypothyroidism	[95]
	Diabetes, myopathy	[96]
A3260G	Myopathy and cardiomyopathy	[88,89]
	MELAS	[87]
T3271C	MELAS	[86,97]
	Deafness and muscular fatigue	[97]
T:A bp deletion (3271–3273)	Encephalopathy, endocrinopathybrain calcification (Fahr syndrome)	[98]
T3291C	MELAS	[28]
A3302G	Myopathy with respiratory failure	[99]
C3303T	Cardiomyopathy	[100]

termination is impaired in vivo [81], or in cytoplasmic hybrids virtually homoplasmic for the MELAS mutation [10,82]. However, a study of patient derived myoblast clones with 93% of the MELAS mutation, showed a decreased steady-state amount of the 16S rRNA when compared with myoblasts harbouring a deletion of mtDNA which did not involve the transcription termination site, suggesting that the capacity to enhance upstream transcription may have been limited by the MELAS mutation [83]. It also remains unclear whether alterations in the processing of RNA 19, a polycystronic precursor transcript corresponding to the 16S rRNA+ $tRNA^{Leu(UUR)}$+ND1 which was found to be increased in MELAS tissues [84], contributes to the markedly reduced rates of synthesis and steady-state levels of mtDNA encoded polypeptides observed in cybrids harbouring $>90\%$ mutant genomes [10,84, 85]. The mtDNA encoded polypeptides with the highest proportion of leucine (UUR) residues are the Complex I subunits ND3 and ND6. Although the functions of these subunits are not known, loss of ND3, ND6 or both might explain why Complex I deficiency is the commonest respiratory chain defect in MELAS muscle [77]. In a recent study of cybrid clones harbouring different proportions of the MELAS 3243 mutation, those containing 60% or more of mutant mtDNAs synthesised little or no ND6 and showed a defect in Complex I [16]. However, different clones harbouring similar levels of mutant mtDNA showed widely differing oxygen consumption rates, and even cybrids with 95% of mutated mtDNA retained some respiratory capacity and COX activity.

Of the 11 additional mutations identified in the $tRNA^{Leu(UUR)}$ gene, 3 have been associated with MELAS [28,86,87] and one of these mutations, an A3260G transition, has also been identified in two families with maternally inherited myopathy and cardiomyopathy [88,89] (Table 3). The remaining $tRNA^{Leu(UUR)}$ mutations have been associated with diverse clinical phenotypes [90–100]. Mutations in 12 other mitochondrial tRNA genes and a unique 9-bp insertion into a non-coding sequence of

Table 4
MtDNA mutations in other tRNA genes (maternal or sporadic)

Gene	Mutation	Phenotype	Ref.
$tRNA^{Phe}$	A583G	MELAS	[101]
	A606G	Acute rhabdomyolysis	[102]
$tRNA^{Val}$	G1606A	Ataxia, seizures, dementia, deafness, myoclonus, cataract	[103]
	G1642A	MELAS	[29,104]
	A1644T	Adult Leigh's syndrome	[105]
$tRNA^{Ile}$	A4269G	Encephalopathy, fatal cardiomyopathy	[106]
	T4274C	PEO, myopathy, ataxia	[107]
	T4285C	PEO	[108]
	A4295G	Hypertrophic cardiomyopathy	[109]
	G4298A	PEO+MS	[110]
	A4300G	Hypertrophic cardiomyopathy	[111]
	A4317G	Encephalomyopathy, fatal cardiomyopathy	[112]
	C4320T	Encephalopathy, cardiomyopathy	[113]
$tRNA^{Trp}$	5537T insert	Optic atrophy, ataxia, dementia	[114]
		Leigh's syndrome	[114]
	G5549A	Dementia, deafness, ataxia, chorea	[115]
$tRNA^{Asn}$	A5692G	PEO, myopathy, ataxia	[116]
	G5703A	PEO	[95]
$tRNA^{Cys}$	A5814G	MELAS	[117]
$tRNA^{Ser(UCN)}$	A7445G	Non-syndromic deafness	[119]
	7472C insert	Deafness, ataxia, myoclonus neuropathy	[120]
	T7512C	MERRF-MELAS overlap	[118]
$tRNA^{Gly}$	T9997C	Hypertrophic cardiomyopathy	[121]
	A10044G	Fatal infantile multisystem disease	[122]
$tRNA^{Leu(CUN)}$	T12311C	PEO	[123]
	G12315A	PEO	[123]
	A12320G	Myopathy	[125]
$tRNA^{Glu}$	T14709C	Congenital encephalomyopathy	[126]
		Myopathy and diabetes	[127]
$tRNA^{Thr}$	G15915A	Encephalomyopathy	[128]
	A15923G	Fatal infantile multisystem disease	[129]
$tRNA^{Pro}$	C15990T	Myopathy	[130]
9-bp insertion between COX II and $tRNA^{Lys}$		PEO, optic atrophy, ataxia, seizures, deafness, dementia	[31]

mtDNA have also been linked to human disease [29,95,101–131]. These are listed together with their respective phenotypes in Table 4. Three of these mutations have also been associated with MELAS, further highlighting the remarkable locus heterogeneity associated with the MELAS phenotype, which to date has been linked to at least 11 different mtDNA mutations (Tables 1–5 and references therein).

4. Large-scale rearrangements of mtDNA involving both tRNA and protein encoding genes

The relationship between the mtDNA genotype and clinical phenotype is no less complex with large-scale deletions of mtDNA which eliminate both tRNA genes and open reading frames (Table 5). These rearrangements are commonly found in sporadic forms of PEO including KSS [132,133], as well as in Pearson's syndrome [134], a potential precursor of KSS [135]. However, deletions alone, or as part of a family of large-scale mtDNA rearrangements comprising deletion dimers and duplications as well as deletions [136], have been identified in other phenotypes including diabetes mellitus and deafness [137], the Wolfram syndrome [138], ataxic leucodystrophy [139], infantile diarrhoea with villous atrophy [140], MELAS [141], the KSS-MELAS over-

Table 5
Large-scale single mtDNA rearrangements

Defect	Phenotype (inheritance)	Ref.
Single deletion	PEO syndromes (sporadic)	[132,133]
	KSS (sporadic)	[132,133]
	KSS+strokes (sporadic)	[142,143]
	Pearson's syndrome (sporadic)	[134]
	Pearson's syndrome to KSS (sporadic)	[135]
	Leigh's syndrome ± Pearson's syndrome (sporadic)	[144]
	MELAS (sporadic)	[141]
	Wolfram syndrome (sporadic)	[138]
	Ataxia leucodystrophy (sporadic)	[139]
	Infantile diarrhoea, villous atrophy (sporadic)	[140]
	Migraine and strokes (sporadic)	[145]
Tandem duplication	PEO+diabetes (maternal)	[151]
	KSS+diabetes (sporadic)	[143,210]
	PEO+multisystem disease (maternal)	[150]
Deletion-duplication	Diabetes+deafness (maternal)	[137]
	Infantile diarrhoea, villous atrophy (sporadic)	[140]

lap [142,143], Leigh's syndrome [31,144] and migraine with recurrent strokes [145]. In patients with PEO syndromes, there appears to be no clear cut correlation between the proportion of deleted mtDNAs in muscle or the site or extent of the deletion and the severity of the clinical phenotype although some correlation has been demonstrated with the so-called common deletion [146]. However, the levels of deleted mtDNAs in different regions of the central nervous system (CNS) also correlates poorly with the clinical features or with the neuropathology, which in KSS resembles that seen in Leigh's syndrome [143]. Despite being present in low abundance in apparently normal mature oocytes [147], mtDNA deletions are usually sporadic, and with few possible exceptions [148] are not maternally transmitted [149]. This is in contrast to mtDNA duplications, which can be maternally transmitted [150,151] possibly because they are less pathogenic [152].

Studies of cybrids harbouring partially deleted genomes have shown a similar sharp decline in respiratory chain activity and in the synthesis of mtDNA encoded proteins, to that seen with the MERRF 8344 and MELAS 3243 mutations, except that the amount of wild-type mtDNA needed to prevent these changes is somewhat higher ($\sim$40%) [9]. The synthesis of polypeptides of the size predicted for fusion proteins in cybrid clones harbouring a deletion which eliminated five tRNA genes indicated that the missing tRNAs were being transcribed from wild-type genomes. When the level of deleted mtDNAs exceeded $\sim$61%, however, evidence for translational complementation was not observed [9].

5. Defects of mtDNA attributed to nuclear gene mutations

Multiple deletions of mtDNA are either sporadic [153,154] or appear to be inherited as mendelian traits, suggesting that the primary genetic errors reside in nuclear genes [155]. Like most other mtDNA abnormalities, they have been identified in a variety of different phenotypes including sporadic, autosomal dominant and autosomal recessive PEO syndromes [154–158], MNGIE [155,159], recurrent myoglobinuria [160], dilated or hypertrophic cardiomyopathy [161,162], Wolfram syndrome [163], sideroblastic anaemia [164] and in patients with Parkinsonian-like features [153,154] or myopathy alone [153]. Multiple deletions have even been identified in one patient with MERRF [165]. Much lower levels of multiple mtDNA deletions, often only detectable by polymerase chain reaction amplification (PCR), have also been identified in muscle from patients with inclusion body myositis [166] and late onset mitochondrial myopathy [167] as

well as in various tissues from older individuals [168,169].

Linkage studies in seven large pedigrees with autosomal dominant PEO syndromes and multiple deletions have assigned the disease locus to chromosome 10q23.3–24.3 in one Finnish family [170] and to chromosome 3p14.1–21.2 in three of six Italian families [171]. The absence of linkage in three Italian families indicates that the autosomal dominant forms of PEO with multiple deletions exhibits considerable genetic heterogeneity.

Tissue specific depletion of apparently normal mtDNA which has been identified in fatal infantile and more benign childhood myopathies [172,173], fatal infantile hepatopathy [174] and in early onset encephalopathy [175,176] appears to conform to an autosomal recessive trait again suggesting that the primary defects reside in the nucleus [155]. Cell fusion experiments using cultured fibroblasts which were found to express the depletion trait have provided some support for this hypothesis [177]. Depletion of mtDNA, however, appears to be a tissue specific disorder and has not been expressed in fibroblast cultures from other cases [155]. A subsequent study by the same group has shown that early primary myoblast cultures from another patient who died of hepatopathy, had normal levels of mtDNA despite the fact that the muscle from which the myoblasts were derived showed a 73% reduction in the level of mtDNA [178]. Later passages and clonal myoblast cultures, however, showed a progressive decline in the level of mtDNA which was restored when the mtDNA depleted mitochondria were transferred to a mtDNA-less (ρ^0) cell line [178]. In another study of a patient with mtDNA depletion in skeletal muscle, the levels of mtDNA in lymphocytes, cultured fibroblasts and myoblasts as well as in myotubes innervated in culture for up to four months were indistinguishable from controls [176]. Moreover, myoblasts from this patient retained the capacity to restore mtDNA levels to normal after being depleted of mtDNA by short term exposure to ethidium bromide [176]. These findings would suggest that the depletion trait is not due to a defect in the resumption of mtDNA replication after formation of the blastocyst as has been proposed [172], but is expressed only at a later stage of muscle differentiation [155,176,178]. Reduced cross-reacting material for mitochondrial transcription factor A (mtTFA) a protein involved in mtDNA transcription and replication [179] as well as reduced levels of its transcript (mtTFA mRNA) have been reported in muscle expressing the depletion trait but the changes could be secondary to the very low levels of mtDNA [180]. Although mtTFA is essential for the maintenance of mtDNA copy number, the findings in a recent study of genetically engineered mice homozygous or heterozygous for a knockout mtTFA allele would suggest that deficiency of mtTFA is unlikely to be the primary cause of tissue-specific mtDNA depletion syndromes in humans [181]. Mice heterozygous for the knockout allele showed a 50% reduction in mtTFA transcripts and protein and about a 40% reduction in mtDNA copy number which was present in all tissues examined [181]. Only the heart muscle, however, showed significantly reduced activities of the respiratory enzyme complexes containing mtDNA encoded subunits. Foetuses homozygous for the mutant allele died before the 10th day of embryonic life with extensive developmental abnormalities and absence of detectable mtDNA and COX activity in all tissues [181].

6. Defects in nuclear genes

Disorders thought to be due to nuclear gene mutations include the fatal and benign infantile myopathies with primary COX deficiency [182], COX deficient Leigh's syndrome [183] and rare but treatable encephalomyopathies due to severe coenzyme Q_{10} deficiency [184,185]. To date, however, only two nuclear inborn errors of oxidative phosphorylation have been identified at a molecular level [5,186]. The first of these involved a C-to-T transition at nucleotide 1684 in the flavoprotein subunit gene of Complex II, which was identified in two sisters with a Leigh-type encephalopathy who were found to have a severe deficiency of Complex II [5]. The second nuclear gene mutation involved a 5-bp duplication in the 18-kDa Complex I subunit gene which destroyed a phosphorylation site in the carboxy terminus of the 18-kDa protein [186]. It was identified in a male infant with a rapidly progressive encephalopathy but normal lactate levels in the blood and cerebrospinal fluid and a normal muscle biopsy [186].

7. Discussion

The remarkable degree of clinical heterogeneity associated with most of the more common mtDNA mutations, as well as the continued discovery of new mutations and new phenotypes has made it difficult to devise a rational classification of these diseases on the basis of mtDNA analysis alone [187]. Most of the distinctive syndromes such as KSS, MELAS, MERRF and MNGIE as well as Leigh's syndrome and LHON have turned out to be genetically heterogeneous and many of the mutations associated with these disorders also occur with other phenotypes which are clinically quite distinct. This is exemplified by the common MELAS 3243 mutation which accounts for 80% of all MELAS cases but is also found in sporadic and maternally inherited PEO syndromes, maternally inherited diabetes mellitus and in myopathy alone. Moreover, several individual clinical manifestations such as dementia, cerebellar ataxia, seizures, deafness and myopathy span a variety of different mtDNA abnormalities and even those which are cardinal to the definition of distinctive syndromes such as PEO, myoclonus, pigmentary retinopathy, strokes and gastrointestinal dysfunction are not mutation specific. As mitochondria are ubiquitous, it might seem reasonable to assume that all mtDNA mutations can affect all tissues, but there are a few exceptions which have so far stood the test of time. Perhaps the most notable of these is the absence of major CNS disease in patients harbouring one of the primary LHON mutations, even to the extent of being virtually homoplasmic for the mutant genome [38,39]. Although an MS-like illness has been described in some affected LHON females with the 11 778 or the 3460 transitions [36] and a severe and sometimes fatal encephalopathy affected several members of a unique Queensland LHON pedigree harbouring the 14 484 mutation [188,189], in the overwhelming majority of cases, LHON appears to be a highly tissue-specific disease [39]. Autoimmune mechanisms, possibly triggered by aberrant Complex I proteins [190] have been invoked to explain the MS-like illness and the unique Queensland LHON pedigree was found to be homoplasmic for a second mtDNA mutation, a T4160C transition in ND1, which may have served to augment the severity of the phenotype [188]. It is interesting that a branch of this family which lacked CNS disease and expressed a more typical LHON phenotype was also homoplasmic for another mutation in ND1 (A4136G) which was thought to have acted as an intragenic suppressor [188]. So-called secondary mutations in mtDNA protein encoding genes have been reported in other pedigrees with LHON but their role in disease pathogenesis is unclear as many of them occur at low frequency in the normal population [38,39,191].

Another notable exception to the notion that all mtDNA mutations can affect all tissues, is the apparent absence of pigmentary retinopathy in patients harbouring the common MERRF 8344 transition. Degeneration of the retinal pigment epithelium has been associated with several of the more common mtDNA mutations and was a prominent feature in the one reported patient with a G8313A transition in the same $tRNA^{Lys}$ gene [72], but as far as we are aware it has not been found in any patient with MERRF 8344. Although the relative proportions of mutant mtDNA in LHON brain and in MERRF 8344 retinal pigment epithelium are not known, their presence at universally high levels in other tissues would suggest that a given mtDNA mutation can cause a defect in oxidative phosphorylation which is manifest clinically in one type of cell population but not in another, even when present in comparably high amounts. With a different mtDNA mutation, however, the situation may be reversed as has been observed with allelic mutations in the same mitochondrial genes [27,54,55,72]. That this may be the case is illustrated by a recent ^{31}P magnetic resonance spectroscopy study of LHON 11 778 skeletal muscle which despite being unaffected clinically showed an abnormally low phosphorylation potential at rest and a markedly reduced rate of phosphocreatine resynthesis after a period of exercise [192]. Similar, but less severe changes were seen with the 14 484 LHON mutation, but the findings in subjects with the 3460 LHON mutation were relatively normal [192]. The mechanisms which determine whether or not a defect in oxidative phosphorylation disrupts the functional integrity of a cell population sufficiently to become apparent clinically remain obscure, but the capacity to eliminate reactive oxygens, which might further impair the cell's metabolic machinery, is one theoretical possibility [193].

Studies of cybrid clones harbouring mtDNA mutations which alter (point mutations) or eliminate (deletions) tRNA genes have shown that when the proportion of mutant mtDNA reaches a certain critical threshold there is a rapid decline in the synthesis of mtDNA encoded proteins and a corresponding loss of respiratory chain activity [8–10,13,16,64,65]. Although variations in segregation behaviour and in threshold have been observed with different donor or recipient cells [16,64,65,194], the ultimate effect of the mtDNA mutation is translational failure. Decreased stability and defective aminoacylation of the mutant tRNA which results in premature translational termination are thought to be the mechanisms with the common MERRF 8344 mutation [66] and a small decrease in aminoacylation has also been reported with the A4317G mutation in the mitochondrial $tRNA^{Ile}$ gene originally identified in fatal infantile cardiomyopathy [195]. The molecular basis for translation failure with other tRNA mutations, however, remains unknown but faulty processing of precursor transcripts, altered post-transcriptional modification and/or stability of the tRNA molecule or impaired recognition by other components of the translational machinery are some theoretical possibilities.

In addition to the mutation itself and its relative abundance with respect to wild-type genomes, there is growing evidence that a multiplicity of other factors are likely to be involved in determining phenotypic expression at a cellular level. Perhaps the most important of these are the overall mtDNA copy number [64,65,194], the mtDNA haplotype [33,194,196], the distribution of mutant and wild-type mtDNAs among the mitochondrial population [64,65,194], the capacity for mitochondrial fusion and for intramitochondrial complementation [197] and the functional reserve or efficiency of the mitochondrial translational machinery [198,199] as well as the nuclear genetic background [64,194]. The importance of the nuclear genetic background was recently demonstrated in lymphoblastoid cell lines derived from members of an Arab-Israeli family with maternally inherited non-syndromic deafness who harboured the A1555G mutation in the 12S rRNA [35]. This study showed that the severity of mitochondrial dysfunction in lymphoblastoid cells homoplasmic for the mutation correlated with the presence or absence of hearing loss in the donor individual [35]. As predicted from studies of other families with the 1555 mutation in which the deafness was induced or enhanced by treatment with aminoglycoside [37,200], the lymphoblast cell lines from both symptomatic and asymptomatic members of this family were found to be markedly sensitive to aminoglycosides when added to the culture medium [35].

At a clinical level the diversity of phenotypes is thought to depend on variations in the degree of mtDNA heteroplasmy in the different tissues and on the threshold levels of mutant mtDNA required to cause cellular or organ dysfunction [1,3]. The multiple factors which are likely to set the level of the threshold effect have already been discussed, but the mechanisms which determine mtDNA heteroplasmy are even more complex. In an individual who maternally inherits a pathogenic mtDNA mutation, the degree of mtDNA heteroplasmy in the tissues of that individual is thought to be determined by the dose of mutant mtDNA in the fertilised egg and the way in which mutated and wild-type genomes replicate and segregate during development. Replication of mtDNA is regulated by nuclear genes, but mitotic segregation is thought to occur by a stochastic or random process [201,202]. However, in a recent study of genetically engineered heteroplasmic mice carrying two different mtDNA genotypes, there was evidence for random genetic drift in some tissues but in liver, kidney, spleen and blood there appeared to be strong tissue-specific selection for different mtDNA genotypes in the same animal [203]. Another study of mtDNA inheritance in the marine mussel *Mytilus edulis* showed that in the male which normally harbours two distinct mtDNAs, both the replication and the partitioning of mitochondrial genomes was selective for the specific male mtDNA genotype [204]. Recent evidence also suggests that the nuclear genetic background may determine the direction of mtDNA segregation in cybrid clones, heteroplasmic for the MELAS 3243 mutation [205]. These studies taken together would suggest that replicative segregation of mtDNA may not be stochastic as has been previously supposed [201,202] but may be determined by a level of interaction between the two genomes, nuclear and mitochondrial, which may vary in different tissues and may encompass genes not directly involved in the formation and maintenance of the oxidative phosphorylation system [206]. In conjunction

with other factors discussed earlier, such a mechanism would help to unravel the enigma of genotype-phenotype associations and would, for example, explain why different phenotypes associated with the common MELAS 3243 mutation tend to segregate in different pedigrees [18,19,78], and why they show differences in the distribution of mutant mtDNAs in their biopsied skeletal muscle fibres [207].

Despite breathtaking advances in the field of mitochondrial medicine which have generated more than 2000 original publications, it is still unclear how MELAS 3243, one of the most common pathogenic mtDNA mutations in humans, can cause diseases as far apart clinically as a devastating and rapidly fatal encephalomyopathy in childhood or early adolescence and benign non-progressive ptosis, non-insulin dependent diabetes mellitus or exercise intolerance in late adult life.

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