How Can We Treat Mitochondrial Encephalomyopathies? Approaches to Therapy

Rita Horvath, Grainne Gorman, and Patrick F. Chinnery

Mitochondrial Research Group, School of Neuroscience, University of Newcastle upon Tyne, United Kingdom

Summary: Mitochondrial disorders are a heterogeneous group of diseases affecting different organs (brain, muscle, liver, and heart), and the severity of the disease is highly variable. The chronicity and heterogeneity, both clinically and genetically, means that many patients require surveillance follow-up over their lifetime, often involving multiple disciplines. Although our understanding of the genetic defects and their pathological impact underlying mitochondrial diseases has increased over the past decade, this has not been paralleled with regards to treatment. Currently, no definitive pharmacological treatment exists for patients with mitochondrial dysfunction, except for patients with primary deficiency of coenzyme Q10. Pharmacological and nonpharmacological treatments increasingly being investigated include ketogenic diet, exercise, and gene therapy. Management is aimed primarily at minimizing disability, preventing complications, and providing prognostic information and genetic counseling based on current best practice. Here, we evaluate therapies used previously and review current and future treatment modalities for both adults and children with mitochondrial disease. **Key Words:** Mitochondrial disease, pharmacological therapy, exercise, coenzyme Q10, trials, genetic counseling.

INTRODUCTION

Mitochondrial disorders are a clinically heterogeneous group of diseases that result from deficiencies in cellular energy production. Affected patients suffer from brain, muscle, liver, and heart damage, and the clinical severity is very variable.[1,2] Many gene defects in this group of patients remain unknown.[3] The chronicity and heterogeneity, both clinically and genetically, means that many patients require surveillance follow-up over their lifetime. This often involves multiple disciplines: both physicians (neurologists, cardiologists, diabetologists, and ophthalmologists) and specialist nurses, physiotherapists, and speech therapists, depending on the clinical phenotype.[4]

Although huge advances have been made over the last decade in relation to the function and structure of mitochondria and subsequently the pathogenic mechanisms underlying mitochondrial disease, there is currently no definitive pharmacological treatment for patients with mitochondrial dysfunction,[5] except for patients with primary deficiency of coenzyme Q10. Nonpharmacological treatments are increasingly being investigated, including ketogenic diet, exercise, and gene therapy.[4] Management is aimed primarily at minimizing disability, preventing complications, and providing prognostic information and genetic counseling based on current best practice.[6–9]

FREQUENCY AND NATURAL COURSE OF MITOCHONDRIAL DISEASES

Recent epidemiological studies confirm that 9.2 in 100,000 people have clinically manifest mtDNA disease.[10] These figures suggest that mtDNA disease is a common cause of chronic morbidity and is more prevalent than previously thought. This not only has implications for allocation of health care resources, but also highlights the need to develop new approaches to the clinical management of patients[11] with mitochondrial disease.

Mitochondrial disorders can affect many different organs, with variable severity, and the pattern of clinical presentation is heterogeneous between different mtDNA mutations as well as between individuals within each genotype.[1,3] Recent development and increasing use of special disease rating scales may aid our understanding of the natural course of mtDNA diseases. Hence, as the

Address correspondence and reprint requests to: Prof. Patrick F. Chinnery, Ph.D., F.R.C.Path., Mitochondrial Research Group, School of Neuroscience, University of Newcastle upon Tyne, UK. E-mail: P.F.Chinnery@ncl.ac.uk.

clinical presentation of the originally defined phenotypes widens, so too does our understanding of these disorders and the mutagenic effect of the mtDNA aberrancy.

To increase the number of patients participating in these studies and the reliability of the ongoing clinical trials, international collaboration of specialized centers is needed. Over the last 5 years, a number of multicenter research collaborations have been forged throughout Europe and North-America, and clinical rating scales are under construction through a European Union consortium (see Eumitocombat, at www.eumitocombat.org). These developments will facilitate multicenter trials on larger cohorts of patients, thus helping to measure the effects of possible therapies and providing hope for treatment in the future.

PREVIOUS THERAPEUTIC OPTIONS

In contrast to the rapid progress in our understanding of the biochemical and molecular bases of mitochondrial encephalomyopathies, therapy options for these patients are still very limited. In a systematic review for the Cochrane Collaboration, Chinnery et al.[5] evaluated randomized and quasi-randomized trials comparing pharmacological and nonpharmacological (vitamins and food supplements) treatments, as well as physical training, in improving the symptoms, signs, disability, and quality of life in individuals with mitochondrial disorders. The authors reviewed 678 abstracts using the Cochrane approach. Only six studies fulfilled study design inclusion criteria and were included in the review.

The six studies assessed the effect of four oral agents: coenzyme Q10 (ubiquinone, or CoQ10),[12,13] creatine monohydrate,[14,15] dichloroacetate,[16] and dimethylglycine.[17] There was objective evidence of locomotor functional improvement with CoQ10 and creatine monohydrate, but with conflicting data for both agents. The trials showed no effect when using higher doses of the oral agent, ruling out inadequate dosage as an explanation for the resultant lack of response. Again, the two trials of longer duration showed no effect, possibly indicating that if there is a treatment response to CoQ10 or creatine monohydrate, it is not sustained. Although secondary measures were shown to improve with dichloroacetate, this was not associated with any improvement in physical function. There was no evidence of a positive response to dimethylglycine. No major side effects of these treatments were recorded.

Since the publication of this Cochrane report, another trial showed that the use of dichloroacetate yielded no clinical improvement and resulted in a partially irreversible toxic neuropathy.[18]

In conclusion, there have been very few randomized controlled clinical trials for the treatment of mitochondrial disease. Those that have been performed were short and involved fewer than 20 study participants, with heterogeneous phenotypes. The authors concluded that there is currently no clear evidence supporting or refuting the use of any of these agents in mitochondrial disorders and that further research is needed to establish the role of a wide range of therapeutic approaches. Further studies are ongoing, to develop new pharmacological treatments in specific forms of mtDNA diseases, including at this center and results are eagerly awaited.

SYMPTOMATIC THERAPY

Despite the reported discrepancies in proven benefit of many pharmacological agents and the lack of cure in mtDNA diseases, treatments do exist. We provide a summary of the currently used beneficial symptomatic treatments.

Treatment of neurological complications

Seizures associated with mtDNA disease usually respond to conventional anticonvulsants, and many of the new antiseizure medications are proving beneficial in these disorders. Valproic acid is historically an effective anticonvulsant for partial and generalized seizures and in patients with myoclonus, which is often present in patients with mitochondrial disease. Increasingly, however, valproic acid is avoided in patients with underlying mitochondrial diseases due to recognized complications. It has been shown to inhibit carnitine uptake,[19] potentially exacerbating fatigability, lactic acidosis, and hyperammonemia, as well as triggering fulminant liver failure[20] and symptoms similar to Reye syndrome.

It should be emphasized, that specifically in children with Alpers syndrome or Alpers-like encephalopathy caused by mutations in the *POLG* gene (alias *POLG1*) a fatal hepatic failure often leading to death can be triggered by valproate.[21] The use of valproate should be also restricted in adults carrying *POLG* mutations.[22]

The exact cause of this toxic reaction is not completely understood. Epilepsy is on of the most important factors influencing morbidity and mortality in patients with mutations in the *POLG* gene.[22] Several patients deteriorated significantly and irreversibly after periods of persistent seizure activity, which usually begins with epilepsia partialis continua (simple partial status) in one limb, and then becomes generalized, resulting in status epilepticus. During the seizure period, brain magnetic resonance imaging shows patchy confluent high signal, which changes over time, followed by accelerated cerebral and cerebellar atrophy. The resulting neuronal energy deprivation, neuronal injury, and cell death in turn predispose to further seizures, leading to a progressive epilepsy syndrome that can be fulminant. Although this sequence highlights the need for rapid and aggressive treatment of seizures, the epilepsy is unfortunately refractory. For

preventive seizure control, most patients seem to benefit from the combination of a sodium channel blocker and a benzodiazepine. Phenytoin, levetiracetam, and topiramate have been also effective in patients with *POLG* mutations.[22] Administration of levetiracetam protects against mitochondrial dysfunction in rats, indicating that, in addition to its antiepileptic actions, levetiracetam may have neuroprotective effects.[23]

A ketogenic diet may be carefully considered for treatment of intractable epilepsy related to respiratory chain defects.[24] However, we could not find reliable data about follow-up analysis of heteroplasmy rates of mtDNA mutations after ketogenic diet, and the exact indications and guidelines need to be further evaluated on higher number of patients.

Parkinsonian symptoms were repeatedly described in association with the defects of the mitochondrial DNA polymerase γ gene (*POLG*),[25] and less frequently with mtDNA mutations.[26] This condition usually shows a good response to levodopa therapy. Psychiatric symptoms—particularly depression—may also accompany mitochondrial diseases and can be effectively treated by psychotropic drugs.[9]

Sensorineural hearing loss may be nonsyndromic, occurring in isolation in A1555G mutation in the *MT-RNR1* gene or syndromic, as associated with the A3243G point mutation in *MT-TL1* gene.[27] Hearing loss can be improved by appropriate hearing devices and the use of cochlear implants in mitochondrial diseases.[28] Ptosis and ophthalmoparesis are common findings in mitochondrial disease. Whereas skeletal muscles generally perform limited specific roles, extraocular muscles have to respond over a wider dynamic range.[29,30] Chronic progressive external ophthalmoplegia is often associated with significant visual impairment and measures of visual disability should be included in studies of natural history and treatment of mitochondrial ocular myopathies. Ptosis in patients with chronic external ophthalmoplegia can be ameliorated by surgery, albeit only transiently. In addition to cosmetic gains, surgical management of ptosis is important to avoid secondary complications, such as postural problems, neck pain, headaches, and even amblyopia, and it should be performed when the visual axis is obscured. The use of silicone slings is recommended in any patient with severe ptosis and less than 8 mm of levator function.[31]

The clinical observation of cataracts in mitochondrial cytopathies has led to hypotheses of cataract formation. Although oxidative stress continues to be the leading proposed mechanism of cataractogenesis, genetic mechanisms are gaining increasing popularity. Surgical excision of cataracts in patients with mitochondrial diseases has led to improved visual function and better quality of life.[32]

Treatment of other complications and the role of solid organ transplantation

Manifestations in other organs are also common. Management of symptoms include management of dysphagia, gastroesophageal reflux, chronic dysmotility with delayed gastric emptying, and intestinal pseudo-obstruction related to the involvement of smooth muscle in many mtDNA diseases, as well as pancreatic exocrine insufficiency. Management is supportive and may involve the use of laxative therapies, antiemetics, and enteric feeding.[33] Blood transfusions in severe phases of anemia or pancytopenia in Pearson syndrome caused by single deletion of the mtDNA may help as a short-term therapy; however, the long-term outcome for these patients is usually poor.

The most frequently affected organs are the pancreas and heart. Diabetes mellitus may respond to dietary or pharmacological therapy, but it is recognized that patients may rapidly move from dietary control to implementation of exogenous insulin due to pancreatic β-cell failure. In patients with maternally inherited diabetes and deafness (MIDD), due to the 3243A>G mutation of mitochondrial DNA (mtDNA), diabetes may present with variable phenotypes. The existence of two distinct phenotypes, MIDD1 and MIDD2, were confirmed in a series of 77 patients[34]; the distinction may be related to the severity of the mitochondrial disease. The role of other genetic or environmental factors in the variable phenotype of MIDD remains to be elucidated; however, the appropriate therapy of the diabetes still needs to be addressed by specialized diabetologists.

Heart conduction blocks, requiring pacemaker implantation, are often present in patients with Kearns–Sayre syndrome. Cardiomyopathy is recognized as accompanying several mitochondrial conditions (e.g. MELAS syndrome or mutations of mtDNA) in adults and can be part of the multisystem disease in young children with mitochondrial encephalomyopathies.[35,36] If severe heart symptoms dominate the phenotype and the other organs are relatively spared, heart transplantation maybe an option. To date, the outcome data are limited, but several articles have reported successful outcomes. Cardiac transplantation with a 50% survival rate has been reported in six patients with mitochondrial cardiomyopathy.[37]

Two cases of patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome and successful recipient outcome have been reported in the literature.[38] Four patients with Barth syndrome, an X-linked recessive disease caused by mutations in the tafazzin gene (*TAZ*), with variable clinical findings including heart failure, myopathy, neutropenia, and growth retardation, have undergone successful orthotopic heart transplantation with no increased rate of rejection.[39] Finally, a child with cardiomyopathy associ-

ated with mitochondrial DNA depletion syndrome successfully underwent heart transplantation recently.[40]

Liver failure is the major symptom of mtDNA depletion caused by mutations in *DGUOK* and *MPV17*.[41] Hepatic disease also develops in children with Alpers–Huttenlocher syndrome caused by autosomal recessive *POLG* mutations in a later phase of the disease, often triggered by valproate therapy for seizure control.[22] Liver transplantation has been performed in numerous cases with mitochondrial hepatopathy. A recent review summarizes the clinical presentation and follow-up of 74 patients with *DGUOK* deficiency worldwide.[42] The authors define the criteria by which patients with *DGUOK* deficiency may benefit from liver transplantation, particularly if other organs are relatively spared. Evaluation of this worldwide sample of patients with pathogenic *DGUOK* mutations suggests that the presence or absence of neurological features is the most appropriate tool for predicting survival. In the presence of neurological features, mortality is increased and organ transplantation does not confer increased survival. Children with *POLG* mutations usually show severe neuromuscular symptoms at the time when liver failure develop; the indication for liver transplantation is questionable.[43]

Our opinion is that isolated organ failure warranting transplantation in the context of mitochondrial disease is not an absolute contraindication *per se*, and the decision to proceed to surgery should be on an individual case basis with input from both neurological and transplant disciplines.

PHARMACOLOGICAL THERAPY

Various pharmacological agents have been used in the treatment of mitochondrial diseases and only seven studies fulfill the study design inclusion criteria using the Cochrane approach[5] (Table 1). In clinical practice, however, multiple supportive therapies have been postulated or used (Table 2).

Removal of noxious metabolites

Removal of noxious metabolites as a therapeutic option has been used with varying results. Lactic acidosis is a typical finding in mitochondrial disease, with lactate recognized as being neurotoxic.[18] Several methods have been used to reduce serum lactate. Buffering lactate with bicarbonate has been shown to have a transient therapeutic effect, but is associated with a detrimental exacerbation of cerebral dysfunction *in vivo*.[44] Dichloroacetate can be used to reduce lactic acid levels, but a recent clinical trial showed that there is no clinical improvement, and the side effects (a partially irreversible toxic neuropathy) are unacceptable.[5]

Increased thymidine levels are involved in the disease pathomechanism in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE),[45] due to a defect of the thymidine phosphorylase enzyme. Hemodialysis was performed in two patients without a clear benefit,[46] but a more prominent effect was shown by allogeneic stem cell transplantation.[47] The exact role of an allogeneic stem cell transplantation in the therapy of MNGIE needs to be further evaluated.

Administration of electron acceptors, metabolites, cofactors, and oxygen radical scavengers

Ubiquinone (coenzyme Q10 or CoQ10) is a lipid-soluble component of the cell membranes, where it functions as a mobile electron and proton carrier.[48] Lack of CoQ10 in mitochondria disrupts the flow of electrons from complexes I and II to complex III, which in turn leads to reduced ATP synthesis. Different compounds of CoQ10 are in use for a wide range of mitochondrial patients,[49] as well as for several neurodegenerative conditions.[50] The delivery of CoQ10 to cells and mitochondria is fraught with difficulty, because of its lipophilic properties. In practice, it is recommended that patients use yogurt or other lipophilic substances to improve the uptake of CoQ10. To date, other delivery systems have been developed, such as mitoQ, a conjugation of the lipophilic triphenylphosphonium cation to ubiquinone, which passes through all biological membranes.[51] Although such systems are promising in terms of improving oral bioavailability, several problems still need to be resolved before the clinical application of such compounds.[52]

Primary CoQ10 deficiency is the main cause of disease in a growing number of patients with an underlying defect in CoQ10 biosynthesis, and many of these patients may show a clear benefit from a supplementation with exogenous CoQ10. Supplementation with CoQ10 resulted in clinical improvement in several early reports, with the primary molecular cause not known.[53,54] Muscle strength returned to normal in two brothers described by Di Giovanni et al.[55] in 2001. Furthermore, a repeated muscle biopsy after 8 months of therapy showed improvement in the histological parameters, normalization of CoQ10 levels and reduction in the proportion of fibers undergoing apoptosis.

In the last 3 years, autosomal recessive mutations have been discovered in an increasing number of nuclear genes coding different enzymes of CoQ10 biosynthesis *(COQ2, PDSS1, PDSS2*) in children with combined respiratory chain (RC) deficiency and severe phenotypes (primary CoQ10 deficiencies).[56–58] A recent publication describing the pathomechanism of different primary CoQ10 deficiencies suggests that patients with *COQ2* mutations show more benefit for therapy than do patients with *PDSS2* mutations.[59]

Because there is no known effective therapy in these neurodegenerative disorders and no documented side ef-

Table 1. *Randomized and Quasi-randomized Trials of Four Oral Agents for Treatment of Mitochondrial Disease*

Study*	Mitochondrial disease	Participants, no.	Dosage	Study design	Statistically significant findings
Coenzyme Q10 (ubiquinone)					
Chen et al., 1997[12]	MERRF, MELAS, CPEO with myopathy	8	160 mg/day	Randomized, double-blind cross-over	MRC score†
Muller et al., 1990[13]	CPEO	17	100 mg/day	Double-blind cross-over	None
Creatine					
Tarnopolsky et al., 1997[14]	MELAS, mitochondrial myopathy	7	4–10 g/day	Randomized, cross-over	Handgrip‡; NIDFT‡; post-exercise lactate†
Klopstock et al., 2000[15]	CPEO, mitochondrial myopathy	16	20 g/day or placebo	Randomized, placebo cross-over	None
Dichloroacetate					
De Stefano et al., 1995[16]	KSS, CPEO with myopathy, MELAS, Leigh's syndrome, mitochondrial depletion syndrome	11	50 mg/kg/day (divided doses)	Double-blind placebo	Blood lactate, pyruvate, alanine–rest and exercise†; brain lactate/creatine ratio†; choline/creatine ratio‡; acetylaspartate/creatine ratio†
Kaufmann et al., 2006[18]	MELAS	30	25 mg/kg/day	Double-blind placebo cross-over	No benefit, toxic neuropathy
Dimethylglycine					
Liet et al., 2003[17]	SLSJ–COX deficiency	5	50 mg/kg/day (divided doses) to 5 g/day	Randomized double-blind	None

CPEO = chronic progressive external ophthalmoplegia; KSS = Kearns–Sayre syndrome; MELAS = mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF = myoclonic epilepsy with ragged red fibers; MRC = Medical Research Council; NIDFT = nonischemic dorsiflexion torque; SLSJ–COX = Saguenay–Lac-Saint-Jean cytochrome oxidase deficiency.
*Six of these seven studies[12–17] are covered in a Cochrane review[5]; the Kaufmann et al.[18] study was published later.
†$p < 0.05$.
‡$p < 0.01$.

fects up to 3000 mg/day CoQ10, a trial of high-dose CoQ10 supplementation might be useful in clinical practice.[60] Pathogenic mutations in the aprataxin gene (*APTX*)[61] and very recently in the *CABC1* gene (alias *ADCK3*)[62] were reported in patients with CoQ10 responsive ataxia. In these patients, muscle CoQ10 is mildly decreased, but the RC enzymes usually show normal activities and muscle biopsy does not provide histological evidence of a mitochondrial involvement. Reduction in muscle CoQ10 was detected in 13 out of 135 patients with genetically undefined cerebellar ataxia. Patients with the ataxic form usually require higher doses and respond less dramatically, probably because of irreversible cerebellar damage.[63] Associated symptoms included seizures, developmental delay, mental retardation, and pyramidal signs. These findings confirm the existence of an ataxic presentation of CoQ10 deficiency, which may be responsive to CoQ10 supplementation; however, the real percentage of recessive ataxias related to mutations in CoQ10 related genes is not known. We suggest that the measurement of CoQ10 in recessive ataxia patients might help to select the group of patients for whom mutations in genes involved in CoQ10 metabolism might unveil the primary cause. More important, these patients might benefit from a supplementation with CoQ10; however, high doses (1000–3000 mg/day) might be needed. More research is needed to determine the real role of CoQ10 in recessive ataxias.

The detection of mutations in the electron-transferring flavoprotein dehydrogenase gene (*ETFDH*) in the myopathic form of CoQ10 deficiency[64] was an important finding in the characterization and therapy of muscle CoQ10 deficiency.[65] Patients with *ETFDH* deficiency, also reported in riboflavin-responsive multiple acyl-CoA dehydrogenation deficiency (MADD),[66] usually show a remarkable clinical improvement on riboflavin therapy (100 mg/day). The fact that a low CoQ10 might be responsible, at least in part, for the clinical symptoms has dramatically changed the therapy of the myopathic form of CoQ10 deficiency and of MADD disease, both of

Table 2. *Therapies Proposed for Mitochondrial Diseases*

Therapy	Study	Indication	Effects
Supportive therapy			
Antiepileptics*	Tein et al., 1993[19]; Krähenbühl et al., 2000[20]	Seizures	Valproate inhibits carnitine uptake causing fatigue, hyperammonemia, lactic acidosis, liver failure, Reye-like syndrome
Antiepileptics*	Tzoulis et al., 2006[22]	Seizures	Alpers syndrome (*POLG* gene)—fulminant liver failure
Antiepileptics*	Kollberg et al., 2006[21]	Seizures	*POLG* mutations in adults—liver failure
Levodopa	Luoma et al., 2004[25]	*POLG* mutations	Parkinsonian symptoms
Antidepressants	DiMauro et al., 2006[9]	All mitochondrial conditions	Positive effect on depressive symptoms
Cochlear implants	Sue et al., 1998[28]	A1555G mutation (*MT-RNR1*); A3243G mutation (*MT-TL1*)	Improvement in hearing loss
Blood transfusion	DiMauro et al., 2006[9]	Pearson's syndrome—single deletion	Severe phases of anemia
Treatment of GI symptoms	Hom et al., 2004[33]	All mitochondrial conditions	Supportive management
Cardiac transplant	Santorelli et al., 2002[40]	Mitochondrial depletion syndrome	1 patient
Cardiac transplant	Bhati et al., 2005[38]	Cardiomyopathy in MELAS	2 patients
Cardiac transplant	Mangat et al., 2007[39]	Barth syndrome	4 patients without increased rejection
Cardiac transplant	Bonnet et al., 2001[37]	Mitochondrial cardiomyopathy	3 of 6 transplanted patients alive
Liver transplant	Dimmock et al., 2008[42]	*DGUOK* mutations;	7 of 10 transplanted patients alive
Removal of noxious metabolites			
Buffer lactate with bicarbonate	De Vivo and DiMauro, 1999[44]	All mitochondrial conditions	Exacerbation of cerebral dysfunction
Buffer lactate with DCA	Kaufmann et al., 2006[18]	MELAS	Toxic neuropathy
Reduce thymidine by hemodialysis	Yavuz et al., 2007[46]	MNGIE	No benefit
Reduce thymidine by stem cell transplantation	Hirano et al., 2007[47]	MNGIE	Benefit noted
Pharmacological therapy			
Copper supplementation	Freisinger et al., 2004[70]	*SCO2* mutations	Improvement in HCM only
Coenzyme Q10	Chen et al., 1997[12]	MERRF, MELAS, CPEO	MRC score only
Coenzyme Q10	Muller et al., 1990[13]	CPEO	No improvement
Changing heteroplasmy			
Endurance training	Jeppesen et al., 2006[85]	Single deletion, microdeletion, point mutation-3243A>G; 8344A>G	No shift in mutant mtDNA in muscle; improvement in oxidative capacity
Gene shifting; aerobic training	Taivassalo et al., 1999[81]	G12315A	Shift in wild type/mutant mtDNA
Endurance training—resistance	Taivassalo et al., 2006[86]	Large single mtDNA deletion	No shift in wilt type/mutant mtDNA
Concentric training	Clark et al., 1997[11]		Decrease in proportion of COX negative fibers
Bupivacaine HCl (0.75%)	Andrews et al., 1999[82]	12320A>G point mutation	No functional recovery (5 patients)

(*Table continues*)

Table 2. *Continued*

Therapy	Study	Indication	Effects
Ketogenic diet	Kang et al., 2007[24]	Respiratory chain and PDH complex defects	7 of 14 patients seizure-free, 3 patients reduce seizure frequency by 50%–90%

CPEO = chronic progressive external ophthalmoplegia; DCA = dichloroacetate; GI = gastrointestinal tract; HCM = hypertrophic cardiomyopathy; MELAS = mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF = myoclonic epilepsy with ragged red fibers; MNGIE = mitochondrial neurogastrointestinal encephalomyopathy; PDH = pyruvate dehydrogenase.
*Except valproic acid.

which are allelic disorders. A combination therapy of CoQ10 (500–1000 mg/day) and riboflavin (100 mg/day) led to complete or almost complete recovery of muscle weakness in seven patients, and a recovery of RC deficiency and the normalization of CoQ10 levels in skeletal muscle in one of these cases.[65]

CoQ10 supplementation, in terms of the beneficial effect on the functioning of the electron transport chain, may also have a role in other mitochondrial disorders; however, the exact effectiveness and the recommended doses are not clearly defined as yet.[5] In clinical practice, most mitochondrial patients might try to take CoQ10, and some report benefit, particularly in relation to muscle discomfort and cramps. CoQ10 and its analog, idebenone, have been used widely in the treatment of neurodegenerative diseases. High-dose idebenone therapy has been shown to be efficacious and safe in Friedreich's ataxia, resulting in improved cardiac function and neurological symptoms.[67] The exact effectiveness of this therapy should be investigated on greater numbers of patients in future trials.

In patients with severe exercise intolerance and deficiency of the RC complex III caused by mutations in *MT-CYB*, two artificial electron acceptors (menadiol diphosphate and vitamin C) were used as therapeutic agents. In one patient, a positive effect was observed on ^{31}P magnetic resonance spectroscopy.[68] No beneficial effect was detected in other individuals with similar types of disease.[69]

Various cocktails of vitamins and cofactors are in use for patients with mitochondrial disease, involving riboflavin, thiamine, folic acid, L-carnitine, and creatine monohydrate. All of these natural compounds are presumed harmless at the doses currently prescribed. Except for anecdotal reports, and recognition of diseases caused by a defect in the metabolism of these compounds, no treatment protocols with these cocktails are currently available for mitochondrial disease. Creatine monohydrate was tried in six patients with MELAS and one with undefined mitochondrial disease in a controlled trial,[5] but the results were inconclusive.[14]

Copper (copper histidinate s.c.) supplementation was tried in a few young children with fatal infantile cardioencephalomyopathy caused by mutations in *SCO2* encoding a COX assembly protein needed for the insertion of copper to the COX holoenzyme. An improvement of hypertrophic cardiomyopathy was observed; however, the severe encephalomyopathy did not change.[70] Copper supplementation might be tried in infants carrying *SCO2* mutations in an early phase of the disease, but the beneficial effect on the neuromuscular symptoms and on the quality of life is not proven. This substance has been used in children with Menkes disease without relevant side effects.

Cell culture experiments on fibroblasts of patients with DGUOK deficiency showed an improvement of mtDNA depletion on supplementation with dAMP/dGMP.[71] Similarly beneficial effect was shown in human muscle cells with DGUOK deficiency (R. Horvath, unpublished data), but not in POLG–deficient muscle cells. It is recognized that excessive levels of nucleotides may also lead to pathology, and thus further studies are needed to evaluate the pathomechanism, effectiveness, and possible side effects of these nucleotides before using them in clinical practice.

Defects of RC may lead to an increased production of reactive oxygen species, resulting in apoptosis[72] and further damage to the cell membranes through lipid peroxidation, ultimately accelerating a higher mutation rate of mtDNA mutations. Evidence of oxidative stress has been proposed not only in mitochondrial disease, but also in many neurodegenerative disorders such as Friedreich's ataxia, Huntington's disease, ALS, and Parkinson's disease in which nuclear mutations affect mitochondrial or nonmitochondrial proteins.[73] In an attempt to avoid the effects of reactive oxygen species, several oxygen radical scavengers have been used in many patients including vitamin E, CoQ10, idebenone, and dihydrolipoate.[74]

GENE THERAPY: CHANGING HETEROPLASMY

Gene therapy as used in other Mendelian diseases (gene delivery by viral and non-viral vectors to the affected tissue, immunological reactions) may be implemented for mitochondrial diseases caused by mutations in nuclear genes. For mtDNA-related diseases, however, the underlying pathological mechanisms are more com-

plex (delivery to mitochondria, heteroplasmy), and new and exciting therapies have been and are being developed.

Inhibition of mutant mtDNA replication

Selective inhibition of the replication of mutant type mtDNA using peptide nucleic acids has been used.[75] Delivery of peptide nucleic acids to mitochondria is not efficient,[76] however, and to date is not a viable therapeutic option.

Import of yeast RNAs

Yeast RNA import for gene therapy has been successfully performed in a form of mtDNA disease. Importing yeast RNAs together with specific yeast transport factors into the cytoplasm of human cybrid cells carrying the A8344G MERRF mutation resulted in the recovery of mitochondrial function.[77]

Import of polypeptides into mitochondria

Importing polypeptides into mitochondria theoretically may happen in different ways: by allotopic expression, by xenotopic expression, and by import of restriction endonucleases. None of these methods to date have been used in routine clinical practice.

Selection for respiratory function

Selecting for respiratory function can be triggered by the use of oligomycin,[78] a mitochondrial poison, or by exposure of the cells to ketone bodies.[79] Both put cells under respiratory stress conditions, leading to the death of cells carrying a high rate of a certain mtDNA mutation, whereas cells with a low mutation rate would survive. These techniques showed a selection of wild-type cells in cell culture experiments, but no *in vivo* patient data are available, because of the toxicity of oligomycin in humans. The implementation of a ketogenic diet may be more promising. It has proven to be an effective therapy for seizures in children with intractable epilepsy and respiratory chain and pyruvate dehydrogenase complex defects.[24] Ketogenic diet may provide similar conditions *in vivo* to ketone bodies by reducing glutamate-induced free radical formation by increasing the NAD^+/NADH ratio and enhancing mitochondrial respiration in neocortical neurons.[80] This mechanism may, in part, contribute to the neuroprotective activity of ketones by restoring normal bioenergetic function in the face of oxidative stress. However, we were unable to find reliable data about follow-up analysis of heteroplasmy rates of mtDNA mutations after ketogenic diet, and the exact indications and guidelines need to be further evaluated on higher number of patients.

Inducing muscle regeneration

Inducing muscle regeneration might lead to the activation of satellite cells into existing muscle fibers. Because satellite cells frequently carry a lower rate of mutant or sometimes no mutation at all, this technique may prove useful in reducing the heteroplasmy of a pathogenic mutation by gene shifting.[81] Muscle regeneration can be induced by injections of substances into muscle (bupivacaine)[11,82] or by isometric exercise causing microtraumas.[84] In support of this, in one patient carrying a nonsense mutation in the COX I gene, recurrent myoglobinuria led to a positive selection of COX positive fibers harboring no mutant mtDNA.[83]

EXERCISE

Exercise is perhaps the most exciting postulated treatment for people with mitochondrial myopathies, particularly those with a muscle phenotype. Moderate exercise is important for patients with mtDNA disease to prevent or reverse the deconditioning that is common in these disorders. Two modes of action are postulated in patients with high mutational loads in muscle: improving exercise intolerance by endurance training and satellite cell activation by resistance training.[84]

Endurance training

Endurance training has been proven to be well tolerated and safe in patients with heteroplasmic mtDNA mutations. Jeppesen et al.[85] looked at 20 patients with either a single deletion (5 cases), microdeletion (1 case), or point mutation (m.3243A>G, 13 cases; m.8344A>G, 1 case) who underwent 12 weeks of endurance training. Although there was an improvement in oxidative capacity, there was no concomitant shift in mutated mtDNA in muscle. Taivassalo et al.[86] looked at eight patients with a large single mtDNA deletion after 14 weeks of endurance training and found similarly no change in the level of muscle deleted mtDNA. Improvements seen in oxygen utilization and extraction, peak work capacity, and submaximal exercise tolerance were maintained in a subset of four patients who continued training for an additional 24 weeks; cessation in training resulted in a loss of gained physiological parameters.[86] As conceded by the authors, additional longer term studies are required not only in this population of mtDNA mutations but in other mitochondrial myopathies with defined genetic cause.

Resistance training

Satellite cells are undifferentiated, myogenic cells known to proliferate in response to muscle fiber injury to replace or repair the damaged muscle fibers. Resistance training using either shortening contractions (concentric) leading to muscle hypertrophy or lengthening contractions (eccentric) leading to segmental muscle necrosis has been proven to induce sufficient traumatic injury to stimulate these satellite cells to be incorporated into the new muscle fibers, a process referred to as *gene shifting*.[81] It is postulated that stimulation of muscle regeneration in this manner may lead to a normal mtDNA

genotype in the regenerated fibers. Preliminary studies injecting bupivacaine hydrochloride (0.75%) to induce muscle necrosis showed satellite cell proliferation exclusively responsible for the derivation of new muscle fibers and an improvement in biochemical activity.[11,82] Taivassalo et al.[86] found an unequivocal decrease in the proportion of COX-negative fibers after concentric training, with an absolute reduction in the number of fibers with mutant mtDNA.

GERMLINE THERAPY

Germline therapy to prevent mtDNA disease transmission and genetic counseling are perhaps the most controversial and emotive elements in the management and treatment of mitochondrial diseases. Development in this area has been rapid over the last 8 to 10 years. The difficulty with many aspects of germline therapy is the difficulty that still remains in prediction of pregnancy outcome, due to the varying mutant load that may pass from mother to child with each pregnancy,[87] and the concern surrounding mutational loads in chorionic and villous sampling.

There are different ways to help mothers carrying mtDNA mutations.[88] A prenatal genetic diagnosis of the fetal DNA might be performed from chorionic villus or by amniocentesis; however, it is questioned whether the mutation rate detected in the sample would reliably reflect the mutation rate in other fetal tissues. The present evidence suggests that this method might be used, with some limitations. More information on heteroplasmy in different fetal tissues will help to evaluate the usefulness of this technique.

Preimplantation genetic diagnostics

With preimplantation genetic diagnostics, it is possible to perform the molecular analysis of the mutation in the polar body from the oocytes or in one to two single cells from an early embryo before implantation of the embryos into the uterus. To date, there is only one report of successfully completed preimplantation genetic diagnostics in mitochondrial disease.[89]

Cytoplasmic transfer

Cytoplasmic transfer between human oocytes has been previously used to improve the success rate of assisted reproduction,[90] and might lead to a decrease in the rate of mutant mitochondria. However, because of the inherent uncertainty and the low effectiveness of this method, it is not likely to become practicable.

Pronuclear transfer

Pronuclear transfer is a technique that could be used to prevent the transmission of an mtDNA disease. It involves the transfer of nuclear DNA from an oocyte with mutant DNA into an enucleated oocyte from a female with normal mtDNA.[91] This may result in a healthy offspring with all the characteristics of the parents (nuclear genes) but without the mutant mtDNA. The most promising pronuclear transfer is between single cell zygotes.[88,92]

FOR THE FUTURE

Long-term follow-up, standardized examination of patients,[93,94] and development of international collaboration to facilitate multicenter trials on larger patient cohorts would offer hope for the future and a chance to possibly modify the natural history of mitochondrial diseases.

REFERENCES

1. Zeviani M, Carelli V. Mitochondrial disorders. Curr Opin Neurol 2007;20:564–571.
2. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med 2003;348:2656–2668.
3. Schon EA, DiMauro S. Mitochondrial mutations: genotype to phenotype. Novartis Found Symp 2007;287:214–225.
4. DiMauro S, Mancuso M. Mitochondrial diseases: therapeutic approaches. Biosci Rep 2007;27:125–137.
5. Chinnery P, Majamaa K, Turnbull D, Thorburn D. Treatment for mitochondrial disorders. Cochrane Database Syst Rev 2006;(1): CD004426.
6. Chinnery PF, Bindoff LA; European Neuromuscular Center. 116th ENMC International Workshop: the treatment of mitochondrial disorders, 14th–16th March 2003, Naarden, The Netherlands. Neuromuscul Disord 2003;13:757–764.
7. Chinnery PF. New approaches to the treatment of mitochondrial disorders. Reprod Biomed Online 2004;8:16–23.
8. DiMauro S, Mancuso M, Naini A. Mitochondrial encephalomyopathies: therapeutic approach. Ann N Y Acad Sci 2004;1011: 232–245.
9. DiMauro S, Hirano M, Schon EA. Approaches to the treatment of mitochondrial diseases. Muscle Nerve 2006;34:265–283.
10. Schaefer AM, McFarland R, Blakely EL, et al. Prevalence of mitochondrial DNA disease in adults. Ann Neurol 2008;63:35–39.
11. Clark KM, Bindoff LA, Lightowlers RN, et al. Reversal of a mitochondrial DNA defect in human skeletal muscle. Nat Genet 1997;16:222–224.
12. Chen RS, Huang CC, Chu NS. Coenzyme Q10 treatment in mitochondrial encephalomyopathies: short-term double-blind, crossover study. Eur Neurol 1997;37:212–218.
13. Muller W, Reimers CD, Berninger T, Boergen K-P, Frey A, Zrenner E, et al. Coenzyme Q10 in ophthalmoplegia plus: a double blind cross over therapeutic trial. J Neurol Sci 1990;98 Suppl:442 (abstract).
14. Tarnopolsky MA, Roy BD, MacDonald JR. A randomized, controlled trial of creatine monohydrate in patients with mitochondrial cytopathies. Muscle Nerve 1997;20:1502–1509.
15. Klopstock T, Querner V, Schmidt F, et al. A placebo-controlled crossover trial of creatine in mitochondrial diseases. Neurology 2000;55:1748–1751.
16. De Stefano N, Matthews PM, Ford B, Genge A, Karpati G, Arnold DL. Short-term dichloroacetate treatment improves indices of cerebral metabolism in patients with mitochondrial disorders. Neurology 1995;45:1193–1198.
17. Liet JM, Pelletier V, Robinson BH, et al. The effect of short-term dimethylglycine treatment on oxygen consumption in cytochrome oxidase deficiency: a double-blind randomized crossover clinical trial. J Pediatr 2003;142:62–66.
18. Kaufmann P, Engelstad K, Wei Y, et al. Dichloroacetate causes toxic neuropathy in MELAS: a randomized, controlled clinical trial. Neurology 2006;66:324–330.

19. Tein I, DiMauro S, Xie ZW, De Vivo DC. Valproic acid impairs carnitine uptake in cultured human skin fibroblasts: an in vitro model for the pathogenesis of valproic acid-associated carnitine deficiency. Pediatr Res 1993;34:281–287.
20. Krähenbühl S, Brandner S, Kleinle S, Liechti S, Straumann D. Mitochondrial disease represents a risk factor for valproate induced fulminant liver failure. Liver 2000;20:346–349.
21. Kollberg G, Moslemi AR, Darin N, et al. *POLG1* mutations associated with progressive encephalopathy in childhood. J Neuropathol Exp Neurol 2006;65:758–768.
22. Tzoulis C, Engelsen BA, Telstad W, et al. The spectrum of clinical disease caused by the A467T and W748S *POLG* mutations: a study of 26 cases. Brain 2006;129:1685–1692.
23. Gibbs JE, Walker MC, Cock HR. Levetiracetam: antiepileptic properties and protective effects on mitochondrial dysfunction in experimental status epilepticus. Epilepsia 2006;47:469–478.
24. Kang HC, Lee YM, Kim HD, Lee JS, Slama A. Safe and effective use of the ketogenic diet in children with epilepsy and mitochondrial respiratory chain complex defects. Epilepsia 2007;48:82–88.
25. Luoma P, Melberg A, Rinne JO, et al. Parkinsonism, premature menopause, and mitochondrial DNA polymerase γ mutations: clinical and molecular genetic study. Lancet 2004;364:875–882.
26. Horvath R, Kley RA, Lochmüller H, Vorgerd M. Parkinson syndrome, neuropathy, and myopathy caused by the mutation A8344G (MERRF) in $tRNA^{Lys}$. Neurology 2007;68:56–58.
27. Sinnathuray AR, Raut V, Awa A, Magee A, Toner JG. A review of cochlear implantation in mitochondrial sensorineural hearing loss. Otol Neurotol 2003;24:418–426.
28. Sue CM, Lipsett LJ, Crimmins DS, et al. Cochlear origin of hearing loss in MELAS syndrome. Ann Neurol 1998;43:350–359.
29. Yu Wai Man CY, Chinnery PF, Griffiths PG. Extraocular muscles have fundamentally distinct properties that make them selectively vulnerable to certain disorders. Neuromuscul Disord 2005;15:17–23.
30. Yu Wai Man CY, Smith T, Chinnery PF, Turnbull DM, Griffiths PG. Assessment of visual function in chronic progressive external ophthalmoplegia. Eye 2006;20:564–568.
31. Wong VA, Beckingsale PS, Oley CA, Sullivan TJ. Management of myogenic ptosis. Ophthalmology 2002;109:1023–1031.
32. Hutnik CM, Nichols BD. Cataracts in systemic diseases and syndromes. Curr Opin Ophthalmol 1999;10:22–28.
33. Hom XB, Lavine JE. Gastrointestinal complications of mitochondrial disease. Mitochondrion 2004;4:601–607.
34. Guillausseau PJ, Dubois-Laforgue D, Massin P, et al; Mitochondrial Diabetes French Study Group. Heterogeneity of diabetes phenotype in patients with 3243 bp mutation of mitochondrial DNA (maternally inherited diabetes and deafness or MIDD). Diabetes Metab 2004;30:181–186.
35. Holmgren D, Wåhlander H, Eriksson BO, Oldfors A, Holme E, Tulinius M. Cardiomyopathy in children with mitochondrial disease: clinical course and cardiological findings. Eur Heart J 2003;24:280–288.
36. Bindoff L. Mitochondria and the heart. Eur Heart J 2003;24:221–224.
37. Bonnet D, Rustin P, Rötig A, et al. Heart transplantation in children with mitochondrial cardiomyopathy. Heart 2001;86:570–573.
38. Bhati RS, Sheridan BC, Mill MR, Selzman CH. Heart transplantation for progressive cardiomyopathy as a manifestation of MELAS syndrome. J Heart Lung Transplant 2005;24:2286–2289.
39. Mangat J, Lunnon-Wood T, Rees P, Elliott M, Burch M. Successful cardiac transplantation in Barth syndrome: single-centre experience of four patients. Pediatr Transplant 2007;11:327–331.
40. Santorelli FM, Gagliardi MG, Dionisi-Vici C, et al. Hypertrophic cardiomyopathy and mtDNA depletion: successful treatment with heart transplantation. Neuromuscul Disord 2002;12:56–59.
41. Copeland WC. Inherited mitochondrial diseases of DNA replication. Annu Rev Med 2008;59:131–146.
42. Dimmock DP, Dunn JK, Feigenbaum A, et al. Neurological phenotype is an important predictor of long term outcome in mitochondrial DNA depletion resulting from deoxyguanosine kinase deficiency. Liver Transpl 2008 (in press).
43. Horvath R, Hudson G, Ferrari G, et al. Phenotypic spectrum associated with mutations of the mitochondrial polymerase γ gene. Brain 2006;129:1674–1684.
44. De Vivo DC, DiMauro S. Mitochondrial diseases. In: Swaiman KF, Ashwal S, editors. Pediatric neurology: principles & practice, 3rd ed. St Louis: Mosby; 1999:494–509.
45. Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. Science 1999;283:689–692.
46. Yavuz H, Ozel A, Christensen M, et al. Treatment of mitochondrial neurogastrointestinal encephalomyopathy with dialysis. Arch Neurol 2007;64:435–438.
47. Hirano M, Martí R, Casali C, et al. Allogeneic stem cell transplantation corrects biochemical derangements in MNGIE. Neurology 2006;67:1458–1460.
48. DiMauro S, Quinzii CM, Hirano M. Mutations in coenzyme Q10 biosynthetic genes. J Clin Invest 2007;117:587–589.
49. Haas RH. The evidence basis for coenzyme Q therapy in oxidative phosphorylation disease. Mitochondrion 2007;7 Suppl:S136–S145.
50. Shults CW, Haas R. Clinical trials of coenzyme Q10 in neurological disorders. Biofactors 2005;25:117–126.
51. Cochemé HM, Kelso GF, James AM, et al. Mitochondrial targeting of quinones: therapeutic implications. Mitochondrion 2007;7 Suppl:S94–S102.
52. Yamada Y, Akita H, Kogure K, Kamiya H, Harashima H. Mitochondrial drug delivery and mitochondrial disease therapy: an approach to liposome-based delivery targeted to mitochondria. Mitochondrion 2007;7:63–71.
53. Rötig A, Appelkvist EL, Geromel V, et al. Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. Lancet 2000;356:391–395.
54. Van Maldergem L, Trijbels F, DiMauro S, et al. Coenzyme Q-responsive Leigh's encephalopathy in two sisters. Ann Neurol 2002;52:750–754.
55. Di Giovanni S, Mirabella M, Spinazzola A, et al. Coenzyme Q10 reverses pathological phenotype and reduces apoptosis in familial CoQ10 deficiency. Neurology 2001;57:515–518.
56. Quinzii C, Naini A, Salviati L, et al. A mutation in para-hydroxybenzoate-polyprenyl transferase (COQ2) causes primary coenzyme Q10 deficiency. Am J Hum Genet 2006;78:345–349.
57. López LC, Schuelke M, Quinzii CM, et al. Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. Am J Hum Genet 2006;79:1125–1130.
58. Mollet J, Giurgea I, Schlemmer D, et al. Prenyldiphosphate synthase, subunit 1 (PDSS1) and OH-benzoate polyprenyltransferase (COQ2) mutations in ubiquinone deficiency and oxidative phosphorylation disorders. J Clin Invest 2007;117:765–772.
59. Quinzii CM, López LC, Von-Moltke J, et al. Respiratory chain dysfunction and oxidative stress correlate with severity of primary CoQ10 deficiency. FASEB J 2008;22:1874–1885.
60. Ferrante KL, Shefner J, Zhang H, et al. Tolerance of high-dose (3,000 mg/day) coenzyme Q10 in ALS. Neurology 2005;65:1834–1836.
61. Quinzii CM, Kattah AG, Naini A, et al. Coenzyme Q deficiency and cerebellar ataxia associated with an aprataxin mutation. Neurology 2005;64:539–541.
62. Lagier-Tourenne C, Tazir M, López LC, et al. ADCK3, an ancestral kinase, is mutated in a form of recessive ataxia associated with coenzyme Q10 deficiency. Am J Hum Genet 2008;82:661–672.
63. Lamperti C, Naini A, Hirano M, et al. Cerebellar ataxia and coenzyme Q10 deficiency. Neurology 2003;60:1206–1208.
64. Horvath R, Schneiderat P, Schoser BGH, et al. Coenzyme Q10 deficiency may cause isolated myopathy. Neurology 2006;66:253–255.
65. Gempel K, Topaloglu H, Talim B, et al. The myopathic form of coenzyme Q10 deficiency is caused by mutations in the electron-transferring-flavoprotein dehydrogenase (*ETFDH*) gene. Brain 2007;130:2037–2044.
66. Olsen RK, Olpin SE, Andresen BS, et al. *ETFDH* mutations as a major cause of riboflavin-responsive multiple acyl-CoA dehydrogenation deficiency. Brain 2007;130:2045–2054.

67. Di Prospero NA, Baker A, Jeffries N, Fischbeck KH. Neurological effects of high-dose idebenone in patients with Friedreich's ataxia: a randomized, placebo-controlled trial. Lancet Neurol 2007;6: 878–886.
68. Argov Z, Bank WJ, Maris J, et al. Treatment of mitochondrial myopathy due to complex III deficiency with vitamins K3 and C: A 31P-NMR follow-up study. Ann Neurol 1986;19:598–602.
69. Keightley JA, Anitori R, Burton MD, Quan F, Buist NR, Kennaway NG. Mitochondrial encephalomyopathy and complex III deficiency associated with a stop-codon mutation in the cytochrome *b* gene. Am J Hum Genet 2000;67:1400–1410.
70. Freisinger P, Horvath R, Macmillan C, Peters J, Jaksch M. Reversion of hypertrophic cardiomyopathy in a patient with deficiency of the mitochondrial copper binding protein Sco2: is there a potential effect of copper? J Inherit Metab Dis 2004;27:67–79.
71. Taanman JW, Muddle JR, Muntau AC. Mitochondrial DNA depletion can be prevented by dGMP and dAMP supplementation in a resting culture of deoxyguanosine kinase-deficient fibroblasts. Hum Mol Genet 2003;12:1839–1845.
72. Beal MF. Mitochondria take center stage in aging and neurodegeneration. Ann Neurol 2005;58:495–505.
73. Mancuso C, Scapagini G, Currò D, et al. Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders. Front Biosci 2007;12:1107–1123.
74. Rodriguez MC, MacDonald JR, Mahoney DJ, Parise G, Beal MF, Tarnopolsky MA. Beneficial effects of creatine, CoQ10, and lipoic acid in mitochondrial disorders. Muscle Nerve 2007;35:235–242.
75. Taylor RW, Chinnery PF, Turnbull DM, Lightowlers RN. Selective inhibition of mutant human mitochondrial DNA replication in vitro by peptide nucleic acids. Nat Genet 1997;15:212–215.
76. Chinnery PF, Taylor RW, Diekert K, Lill R, Turnbull DM, Lightowlers RN. Peptide nucleic acid delivery to human mitochondria [Erratum in: Gene Ther 2000;7:813]. Gene Ther 1999;6:1919–1928.
77. Kolesnikova OA, Entelis NS, Jacquin-Becker C, et al. Nuclear DNA-encoded tRNAs targeted into mitochondria can rescue a mitochondrial DNA mutation associated with the MERRF syndrome in cultured human cells. Hum Mol Genet 2004;13:2519–2534.
78. Manfredi G, Gupta N, Vazquez-Memije ME, et al. Oligomycin induces a decrease in the cellular content of a pathogenic mutation in the human mitochondrial ATPase 6 gene. J Biol Chem 1999; 274:9386–9391.
79. Santra S, Gilkerson RW, Davidson M, Schon EA. Ketogenic treatment reduces deleted mitochondrial DNAs in cultured human cells. Ann Neurol 2004;56:662–669.
80. Maalouf M, Sullivan PG, Davis L, Kim DY, Rho JM. Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. Neuroscience 2007;145:256–264.
81. Taivassalo T, Fu K, Johns T, Arnold D, Karpati G, Shoubridge EA. Gene shifting: a novel therapy for mitochondrial myopathy. Hum Mol Genet 1999;8:1047–1052.
82. Andrews RM, Griffiths PG, Chinnery PF, Turnbull DM. Evaluation of bupivacaine-induced muscle regeneration in the treatment of ptosis in patients with chronic progressive external ophthalmoplegia and Kearns–Sayre syndrome. Eye 1999;13:769–772.
83. Karadimas CL, Greenstein P, Sue CM, et al. Recurrent myoglobinuria due to a nonsense mutation in the COX I gene of mitochondrial DNA. Neurology 2000;55:644–649.
84. Gardner JL, Craven L, Turnbull DM, Taylor RW. Experimental strategies towards treating mitochondrial DNA disorders. Biosci Rep 2007;27:139–150.
85. Jeppesen TD, Schwartz M, Olsen DB, et al. Aerobic training is safe and improves exercise capacity in patients with mitochondrial myopathy. Brain 2006;129:3402–3412.
86. Taivassalo T, Gardner JL, Taylor RW, et al. Endurance training and detraining in mitochondrial myopathies due to single large-scale mtDNA deletions. Brain 2006:129:3391–3401.
87. Brown DT, Herbert M, Lamb VK, et al. Transmission of mitochondrial DNA disorders: possibilities for the future. Lancet 2006; 368:87–89.
88. Gardner DK, Sheehan CB, Rienzi L, Katz-Jaffe M, Larman MG. Analysis of oocyte physiology to improve cryopreservation procedures. Theriogenology 2007;67:64–72.
89. Steffann J, Feyereisen E, Kerbrat V, Romana S, Frydman N. Prenatal and preimplantation genetic diagnosis: decision tree, new practices? [In French]. Med Sci (Paris) 2005;21:987–992.
90. Cohen J, Scott R, Schimmel T, Levron J, Willadsen S. Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. Lancet 1997;350:186–187.
91. Roberts RM. Prevention of human mitochondrial (mtDNA) disease by nucleus transplantation into an enucleated donor oocyte. Am J Med Genet 1999;87:265–266.
92. McGrath J, Solter D. Nuclear transplantation in the mouse embryo by microsurgery and cell fusion. Science 1983;220:1300–1302.
93. Schaefer AM, Phoenix C, Elson JL, McFarland R, Chinnery PF, Turnbull DM. Mitochondrial disease in adults: a scale to monitor progression and treatment. Neurology 2006;66:1932–1934.
94. Phoenix C, Schaefer AM, Elson JL, et al. A scale to monitor progression and treatment of mitochondrial disease in children. Neuromuscul Disord 2006;16:814–820.