

# Clinical Spectrum and Diagnosis of Mitochondrial Disorders

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Respiratory chain deficiencies have long been regarded as neuromuscular diseases mostly originating from mutations in the mitochondrial DNA. Actually, oxidative phosphorylation, i.e., adenosine triphosphate (ATP) synthesis-coupled electron transfer from substrate to oxygen through the respiratory chain, does not only occur in the neuromuscular system. For this reason, a respiratory chain deficiency can theoretically give rise to any symptom, in any organ or tissue, at any age and with any mode of inheritance, owing to the dual genetic origin of respiratory chain enzymes (nuclear DNA and mitochondrial DNA). In recent years, it has become increasingly clear that genetic defects of oxidative phosphorylation account for a large variety of clinical symptoms in both childhood and adulthood. Diagnosis of a respiratory chain deficiency is difficult initially when only one symptom is present, and easier when additional, seemingly unrelated, symptoms are observed. The clinical heterogeneity is echoed by the genetic heterogeneity illustrated by the increasing number of nuclear genes that have been shown to be involved in these diseases. In the absence of clear-cut genotype-phenotype correlations and in front of the large number of possibly involved genes, biochemical analyses are still the cornerstone of the diagnosis of this condition. © 2001 Wiley-Liss, Inc.

**KEY WORDS:** mitochondrial disease; clinical presentation; nuclear DNA; mitochondrial DNA; polarography; spectrophotometry

## THE UNDERLYING CELL DYSFUNCTION

Oxidative phosphorylation, i.e., ATP synthesis by the oxygen-consuming respiratory chain (RC), supplies most organs and tissues with energy, being already functional before birth [Tyler, 1992]. Consequently, RC deficiency can theoretically give rise to any symptom, in any organ or tissue, at any age, with any mode of inheritance, due to the twofold genetic origin of RC components (nuclear DNA and mitochondrial DNA; nDNA and mtDNA). Addition-

ally, RC dysfunction can originate from non-genetic causes and several confounding factors may aggravate clinical outcome (Fig. 1).

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In the last few years, it has become increasingly clear that defects of oxida-

tive phosphorylation account for a large variety of clinical symptoms in both childhood and adulthood. Overall, the diagnosis of RC deficiency is difficult to consider when the first symptom occurs. The diagnosis becomes easier when two seemingly unrelated symptoms are observed.

The RC catalyzes the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP via five complexes embedded in the inner mitochondrial membrane (Fig. 2). Complex I (NADH-ubiquinone reductase) carries reducing equivalents from NADH to the ubiquinone pool (coenzyme Q; CoQ) and consists of 43 polypeptides, seven of which are encoded by mtDNA. Complex II (succinate-ubiquinone reductase) carries reducing equivalents from FADH<sub>2</sub> to the CoQ pool. It contains the two subunits of the succinate dehydrogenase (flavoprotein and iron-sulfur subunits) plus two anchoring subunits (subunits C and D) that bind a *b*-type cytochrome. This is the only RC complex that does not contain any mtDNA-encoded protein. Additional proteins, e.g., the electron transfer flavoprotein (ETF), glycerol-3-phosphate dehydrogenase,

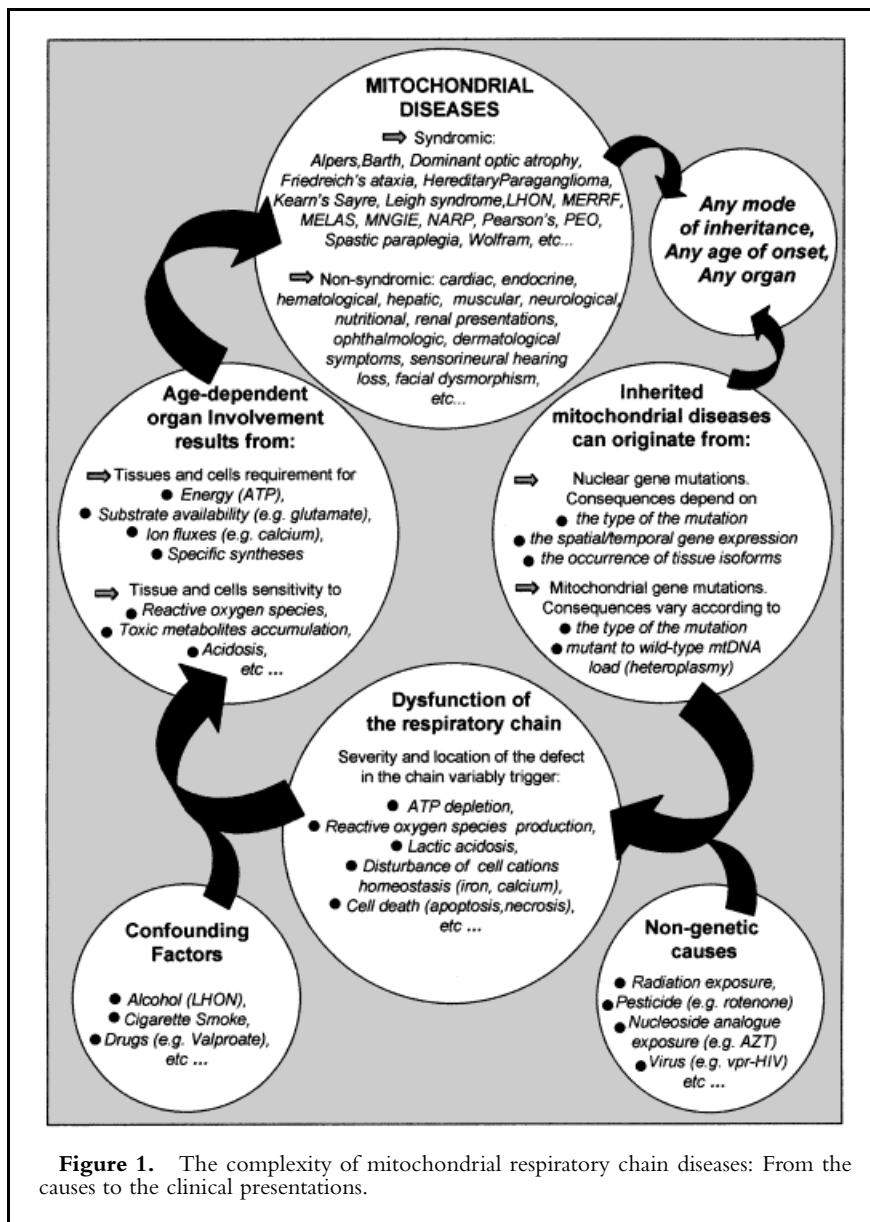
Arnold Munnich, M.D., Ph.D., is professor at the University René Descartes (Paris V) and is the founder of the Department of Genetics, Hôpital des Enfants-Malades, Paris, which brings together i) a Research unit (INSERM U393) dedicated to the mapping and identification of genes causing developmental and neurogenetic diseases in children, and ii) the Medical Genetic Clinic of Assistance Publique, Hôpitaux de Paris in the Hôpital Necker-Enfants Malades in Paris. The originality of his project consists in the combination of a clinical expertise and a molecular approach of medical genetics in the unique environment of a large European pediatric hospital.

Pierre Rustin, Ph.D., is Research Director at the CNRS (Centre National de la Recherche Scientifique) in charge, with Dr. Agnès Rötig, of the Research group working on mitochondrial diseases in children in the Research unit (INSERM U393) of the Hôpital Necker-Enfants Malades in Paris. He spent ten years studying interactions between mitochondria and chloroplasts in plants at the Université Pierre et Marie Curie, Paris, before Arnold Munnich invited him, in 1988, to join the Department of Genetics to focus his activity on mitochondrial diseases in children. His research group brings together the clinical, molecular genetic, and biochemical competencies often essential for the comprehension of these multifaceted diseases.

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**Figure 1.** The complexity of mitochondrial respiratory chain diseases: From the causes to the clinical presentations.

should result in one or more of: 1) an increase of reducing equivalents in both mitochondria and cytosol (e.g., elevated NADH/NAD<sup>+</sup> ratio), 2) a decrease in mitochondrial ATP formation, 3) an increase of monovalent reduction of oxygen, resulting in superoxide formation, and 4) the functional impairment of numerous metabolic pathways requiring RC function, e.g., the tricarboxylic acid cycle and  $\beta$ -oxidation (Fig. 2). Therefore, an increase of ketone body ( $\beta$ -OH butyrate/acetoacetate) and lactate/pyruvate (L/P) molar ratios with secondary elevation of blood lactate might be found in the plasma of affected individuals [Robinson, 1995]. This is particularly true in the post-absorptive period, when more NAD<sup>+</sup> is required for adequately oxidizing glycolytic substrates. Similarly, as a consequence of the functional impairment of the citric acid cycle, ketone body synthesis increases after meals due to the channeling of acetyl CoA towards ketogenesis. The elevation of total ketone bodies in a fed individual is *paradoxical*, as it should normally decrease after meals, due to insulin release (paradoxical hyperketonemia) [Munnich et al., 1996a].

## THE CLINICAL PRESENTATIONS

A defect of oxidative phosphorylation can be suspected in patients presenting with 1) an unexplained combination of neuromuscular and/or non-neuromuscular symptoms, 2) a progressive course, and 3) involvement of seemingly unrelated organs or tissues. The clinical symptoms, either isolated or in combination, may occur at any stage, but a frequent feature is the increasing number

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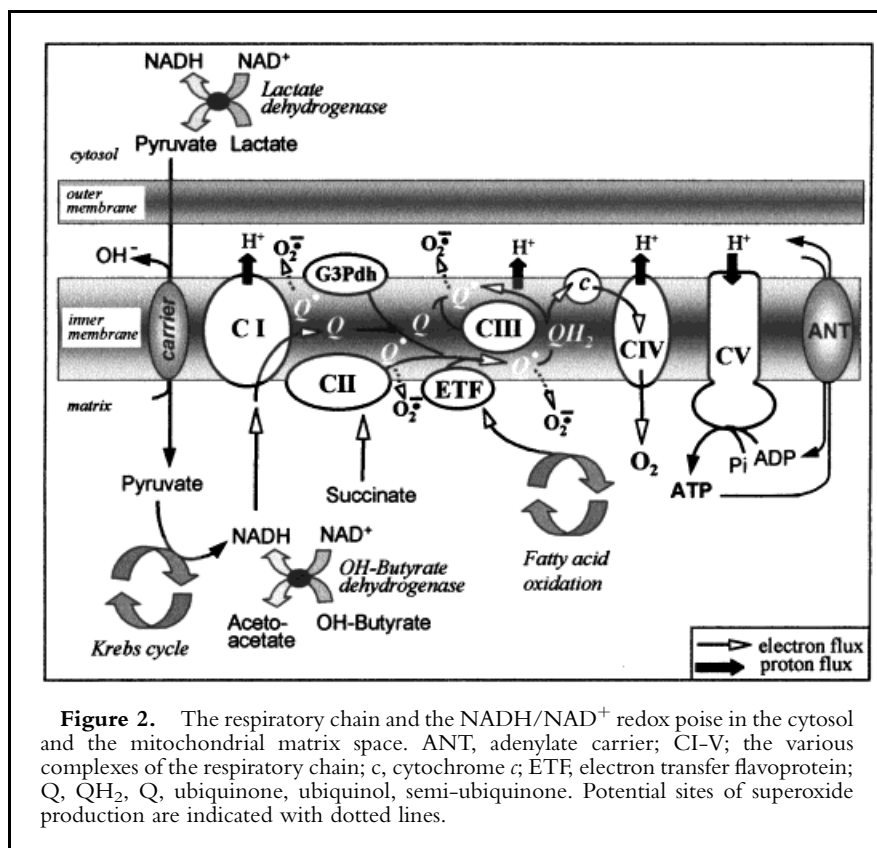
of organs involved in the course of the disease. The progressive organ involvement occurs regardless of age at onset

and dihydroorotate dehydrogenase, also compete to feed electrons to the CoQ pool. Complex III (ubiquinol-cytochrome *c* reductase) carries electrons from the CoQ pool to cytochrome *c*. It contains 11 subunits, one of which (cytochrome *b*) is encoded by mtDNA. Complex IV (cytochrome *c* oxidase, COX), the terminal oxidase, catalyzes the transfer of reducing equivalents from cytochrome *c* to molecular oxygen, producing water. It contains two cytochromes (*a* and *a*<sub>3</sub>), two copper atoms, and 13 subunits, three of which are encoded by mtDNA [Tzagoloff, 1982].

CoQ, a highly hydrophobic quinone, and cytochrome *c*, a low molecular

weight hemoprotein, act as "shuttles" between complexes. The free energy generated from the redox reactions is converted into a transmembrane proton gradient. Protons are pumped through complexes I, III, and IV of the RC, which creates a charge differential. Complex V (ATP synthase) allows protons to flow back into the mitochondrial matrix and uses the released energy to synthesize ATP. Three ATP molecules are made for each NADH oxidized.

Since the RC catalyzes the divalent reduction of molecular oxygen to water with concomitant ATP production, a disorder of oxidative phosphorylation



**Figure 2.** The respiratory chain and the NADH/NAD<sup>+</sup> redox poise in the cytosol and the mitochondrial matrix space. ANT, adenylate carrier; CI-V; the various complexes of the respiratory chain; c, cytochrome c; ETF, electron transfer flavoprotein; Q, QH<sub>2</sub>, Q, ubiquinone, ubiquinol, semi-ubiquinone. Potential sites of superoxide production are indicated with dotted lines.

and clinical presentation. Yet, while the initial symptoms usually persist and gradually worsen, they may occasionally improve or even disappear, as other organs become involved.

### Neurological Presentation

The central and/or peripheral nervous system is frequently involved in mitochondrial disorders. The disease may start in the neonatal period with drowsiness, poor sucking, severe hypotonia, abnormal movements, seizures, respiratory distress, and fatal ketoacidotic coma with major lactic acidosis. Severe encephalopathy may start later in childhood after a period of apparently normal development. Frequently, the first cause of concern is poor head control, an inability to roll over, and a delay in sitting without support or walking unaided. Later, the clinical profile results from the variable combination of the following neurological symptoms: trunk hypotonia, cranial nerve and brainstem involvement (abnormal eye movements, ophthalmoplegia, recurrent apneas), cerebellar ataxia, myoclonia, seizures,

pyramidal syndrome, peripheral neuropathy, poliodystrophy and leukodystrophy. These patients often present with bouts of drowsiness and frequently get worth following intercurrent infections [Ogier and Aicardi, 1992; DiMauro and Bonilla, 1993].

Leukodystrophy, i.e., the diffuse impairment of the cerebral white matter, predominantly results in motor disturbance with slow mental retardation and low incidence of seizures. Several syndromes of RC deficiency also involve the gray matter, including mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) and Leigh syndromes (see below) [Valanne et al., 1998]. Diffuse involvement of the central white matter can also occur as the major or even unique manifestation of the disease and RC deficiency should be regarded, therefore, as a possible cause of isolated leukodystrophy in childhood.

### Muscular Presentation

Various degrees of muscle involvement are observed, ranging in severity from

fatal infantile myopathy to progressive muscle weakness in childhood and adulthood. Infantile myopathy presents with severe progressive and generalized weakness, respiratory distress and lactic acidosis. Patients with the fatal form die before one year of age of respiratory failure and multiple organ involvement [Shoffner and Wallace, 1995]. Yet, spontaneous remissions of infantile myopathy have been observed despite severe initial weakness [DiMauro et al., 1983].

The myopathic form is characterized by hypotonia and progressive weakness of the limbs, with exertion intolerance and muscle atrophy. Symptoms may appear within the first two years of life or later in childhood or adulthood. Myopathy may remain isolated or become associated with various additional symptoms such as retinal dystrophies, ophthalmoplegia, or other organ involvement. Plasma lactate level at rest is normal or mildly elevated but exercise can trigger a significant elevation of lactic acid.

Other muscular symptoms are occasionally observed, such as myalgias or myoglobinuria. Recently, few cases have been reported with recurrent attacks of trunk and limb hypotonia, myalgias, muscle stiffness, lethargy, and iterative episodes of myoglobinuria, ascribed to RC deficiency [Saunier et al., 1995; Keightley et al., 1996]. Surprisingly, all patients had generalized hyporeflexia during attacks, subnormal plasma lactate levels, and inconstant enzyme deficiency in muscle. Expression of the enzyme deficiency in cultured fibroblasts helps diagnose this condition, especially when rhabdomyolysis precludes skeletal muscle biopsy during and/or after acute episodes. Muscle cell lysis might originate from the release of mitochondrial components (cytochrome c, AIF) that are known to activate the apoptotic program [Kluck et al., 1997; Yang et al., 1997].

### Cardiac Presentation

Genetic defects of oxidative phosphorylation represent a major cause of cardiomyopathy in children. Cardio-

myopathy frequently appears as the onset symptom or as part of a multiorgan involvement [Marin-Garcia and Goldenthal, 1997]. Iterative apneas, dyspnea,

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cyanosis, or bronchitis in the neonatal period may be the presenting symptoms of a subsequently fatal mitochondrial cardiomyopathy. Delayed onset forms are frequently diagnosed in patients with unexplained heart failure [Munnich et al., 1996b]. The redox status in plasma is consistently disturbed in the neonatal onset form but frequently subnormal in the delayed onset form. Most patients have concentric hypertrophic cardiomyopathy and the question of whether the dilated forms are primarily or secondarily dilated is still debated.

Endomyocardial biopsy is a reliable diagnostic tool in cardiomyopathy [Rustin et al., 1994b]. Heart morphology is frequently abnormal (interstitial fibrosis, fibroelastosis). All types of RC enzyme deficiency have been observed, but complex I deficiency is significantly more frequent [von Kleist-Retzow et al., 1998]. Point mutations, as well as multiple mtDNA deletions, have been described in several families [Kogelnik et al., 2000]. Barth syndrome is an important subtype of syndromic cardiomyopathy (see below) [Barth et al., 1983; Bolhuis et al., 1991]. It is an antenatal or neonatal onset cardiomyopathy with cyclic neutropenia and frequently fatal outcome. It is, therefore, important to carefully look at blood counts in boys with neonatal-onset cardiomyopathy, as the recent identification of the disease gene on chromosome Xq28 helps diagnose this condition [Bione et al., 1996]. In addition, the biochemical analysis performed on endomyocardial biopsy is particularly valuable in the significant fraction of cases that are not expressed in other tissues. Indeed, while multiorgan

involvement is usually regarded as a contra-indication of organ transplantation, it is reasonable to consider heart transplantation in slowly progressive forms limited to the myocardium.

### **Renal Presentation**

Renal involvement is not uncommon in RC deficiency. The first symptoms develop in the neonatal period or before the age of two years. The most common manifestation is a proximal tubulopathy with de Toni-Debré-Fanconi syndrome [Rötig et al., 1994; Wendel et al., 1995; Niaudet and Rötig, 1996]. Other renal presentations have been reported, including glomerular disease with a nephrotic syndrome and chronic tubulo-interstitial nephropathy [Rötig et al., 1994; Wendel et al., 1995; Szabolcs et al., 1994; Rötig et al., 1995b]. Plasma lactate and L/P ratios are consistently normal but, interestingly, abnormal urinary lactate and Krebs cycle intermediates point toward RC deficiency. The Fanconi syndrome is characterized by an impairment of proximal tubular re-absorption, leading to urinary loss of amino acids, glucose, proteins, ions, and water. In RC deficiency, it is frequently limited to mild aminoaciduria, and occasionally responsible for metabolic acidosis. When available, the renal biopsy shows unspecific anomalies of the tubular epithelium with dilations or obliteration by casts, dedifferentiation or atrophy, and occasionally giant mitochondria. Glomerular disease with nephrotic syndrome has been reported in patients with pathological evidence of focal and segmental glomerular sclerosis [Rötig et al., 1994]. In addition, tubulo-interstitial nephritis has been occasionally reported in patients with chronic renal insufficiency. Renal biopsy showed diffuse interstitial fibrosis with tubular atrophy and sclerosed glomeruli. Finally, the MELAS mutation has been described in several families with cardiomyopathy, diabetes mellitus, sensorineural deafness and renal failure unrelated to diabetes mellitus [Damian et al., 1995; Hsieh et al., 1996; Jansen et al., 1997; Kogelnik et al., 2000]

### **Nutritional Presentation**

A significant fraction of affected neonates are small for gestational age, a feature that illustrates the frequently antenatal expression of the disease [Tulinius et al., 1995; von Kleist-Retzow, 1998]. Alternatively, post-natal growth failure may occur, at any age, after several months of apparently normal development. The unexplained inflexion of the weight/height curve may long be the unique manifestation of the disease. Severe anorexia, recurrent vomiting, chronic diarrhea with villous atrophy, and/or exocrine pancreatic dysfunction occasionally occur [Rötig et al., 1990; Cormier-Daire et al., 1994]. These features are often ascribed to gluten or cow-milk protein intolerance but they do not recover with the appropriate change of diet. These clinical forms are consistently associated with disturbed redox status in plasma and frequently with mtDNA rearrangements in various tissues. Interestingly, while the gastrointestinal symptoms usually persist and gradually worsen, remarkable remissions of watery diarrhea have been reported in infants who later developed other organ involvement [Cormier-Daire et al., 1994]. In adulthood, chronic intestinal pseudo obstruction has been occasionally ascribed to RC deficiency [Hirano et al., 1994; Shoffner and Wallace, 1995].

### **Hepatic Presentation**

Two forms of hepatic failure resulting from genetic defects of oxidative phosphorylation have been recognized on the bases of clinical course and severity: a severe neonatal form (40%) and a delayed-onset form (60%) [Cormier-Daire et al., 1997]. The neonatal form has an early onset (before one week), a rapidly fatal course, and frequently includes severe hypotonia, myoclonus epilepsy, and psychomotor retardation [Edery et al., 1994; Bakker et al., 1996]. The other type has a delayed onset (from 2 to 18 months), a milder clinical course, inconstant neurological involvement, and an occasionally fatal outcome [Cormier-Daire et al., 1997]. The

delayed onset form deserves intensive efforts because, in several cases, spontaneous recovery or marked improvement after ursodeoxycholic therapy and liver transplantation has occurred. It is reasonable to limit liver transplantation to patients with severe hepatic failure but no neurological symptoms or evidence of extra hepatic involvement.

Abnormal histology (steatosis, micro-, and macro-nodular cirrhosis) and elevated plasma or cerebrospinal fluid (CSF) lactate are consistent features of the disease, regardless of the clinical subtype. With regard to extension of the disease, brain was the most severely affected organ, but other organs could be occasionally involved. While mtDNA depletion has been occasionally observed in severe hepatic failure [Bakker et al., 1996], the molecular basis of the disease remains largely unknown [Spelbrink et al., 1998].

### Endocrine Presentation

Endocrine presentation includes dwarfism [Tulinius et al., 1995], diabetes mellitus [Gerbitz et al., 1995], hypoparathyroidism [Tengan et al., 1998], and, rarely, hypothyroidism and ACTH deficiency. Major growth retardation ( $-4$  to  $-6$  SD) with normal basal and stimulated plasma growth hormone but markedly reduced plasma IgF1 has been reported in infancy [Munnich et al., 1996b]. The redox status in plasma is consistently disturbed and a fatal multi-organ involvement, probably precipitated by exogenous growth hormone administration, occurs in the course of the disease.

Diabetes mellitus (either insulin dependent or independent) can be inaugural, but often appears as a complication of the disease. Yet, it is important to be aware that diabetes mellitus can occur at very early stages of the disease. Recently, rare cases of neonatal-onset diabetes mellitus have been ascribed to RC deficiency [Munnich et al., 1996b]. The neonates had major hyperglycemia, hyperlactatemia, ketosis, and widespread RC enzyme deficiency. Mitochondrial diabetes is frequently associated with other symp-

toms: deafness, heart, and renal failure. It is either sporadic [Rötig et al., 1993; Superti-Furga et al., 1993; Poulton et al., 1995; Souied et al., 1998] or maternally inherited [Ballinger et al., 1992; Rötig et al., 1992; van den Ouweland et al., 1992; Dunbar et al., 1993; Kadowaki et al., 1994]. The former usually results from mtDNA rearrangements as part of specific syndromes [Kearns-Sayre syndrome (KSS), Pearson, Wolfram syndrome; see below], the latter is the result of either mtDNA mutations or deletions/duplications. The mtDNA mutation that most commonly causes mitochondrial diabetes is the tRNA<sup>Leu</sup> (UUR) mutation [van den Ouweland et al., 1992], which is also known to cause MELAS, but other mtDNA point mutations have been also reported [Zeviani et al., 1991; Morten et al., 1993; Moraes et al., 1993; Pilz et al., 1994]. Several reported patients (aged 20–40 years) had maternally inherited diabetes mellitus and sensorineural hearing loss but usually none of the other MELAS symptoms and no anti  $\beta$ -islet antibodies [van den Ouweland et al., 1992]. Occasionally however, diabetes mellitus and deafness have been shown to segregate with the complete MELAS syndrome or even multiple organ involvement in the same pedigree [Gerbitz et al., 1995]. In Japan, the prevalence of the tRNA<sup>Leu</sup> mutation is 6% and 0% in familial and sporadic type I diabetes mellitus, respectively, and 2% in familial type II diabetes [Kadowaki et al., 1994]. In France, the prevalence of the mutation in familial type I diabetes is below 2% but this rate averages 60% in patients with combined diabetes mellitus and deafness [Vionnet et al., 1993].

### Hematological Presentation

Childhood myelodysplasia occasionally occurs as the initial symptom of a RC deficiency [Bader-Meunier et al., 1994]. Refractory anemia with ring sideroblasts and vacuolization of marrow precursors is usually associated with variable degrees of neutropenia and thrombopenia, as observed in the Pearson syndrome (see below) [Rötig et al., 1995a; Smith et al., 1995]. The absence of cytogenetic

abnormalities and the polyclonal pattern of peripheral neutrophils and lymphocytes support the view that mitochondrial myelodysplasia should not be regarded as a malignant condition. This clinical presentation emphasizes the difficulty in recognizing the mitochondrial origin of the disease in patients with solely hematological symptoms.

### Sensorineural Hearing Loss

Sensorineural hearing loss is a frequent presentation of mitochondrial diseases. Interestingly, variable degrees of non-syndromic sensorineural hearing loss following amino-glycoside exposure have been described in individuals carrying a homoplasmic mitochondrial tRNA<sup>Ser</sup> (T7445C) [Reid et al., 1994] or 12S rRNA mutation (A1555G) [Prezant et al., 1993; Matthijs et al., 1996; El-Schahawi et al., 1997; Estivill et al., 1998]. The latter mutation is believed to make the rRNA more similar to the bacterial RNA involved in amino-glycoside-induced bactericidal activity and alter translational activity. Yet, several inbred pedigrees have been reported with family members being deaf without drug exposure [Matthijs et al., 1996]. For this reason, a two-hit model of development has been proposed. The incompletely penetrant homoplasmic rRNA 12S mutation is maternally transmitted and represents the first hit, the second hit consisting in either amino-glycoside exposure (non-toxic doses) or homozygosity for an autosomal recessive mutation, altering a putative cochlear-specific rRNA subunit [Fischel-Ghodsian, 1998]. The major clinical relevance of this is the prevention of antibiotic-induced hearing loss, especially as the A1555G mutation accounts for 15% of all cases of amino-glycoside-induced deafness in the USA [Fischel-Ghodsian, 1998]. Physicians should inquire about a family history of antibiotic-induced hearing loss prior to local or general amino-glycoside administration and, conversely, individuals with amino-glycoside-induced hearing loss should be screened for this mutation, as its detection will allow counseling of maternal relatives to

avoid amino-glycosides. More generally, it might be reasonable and cost-effective to screen individuals with non-syndromic hearing loss for the mutation unless maternal inheritance has been excluded [Fischel-Ghodsian, 1998].

### Ophthalmologic Symptoms

The ophthalmologic manifestations of RC deficiency are numerous. They frequently occur in the course of the disease, occasionally as part of well-identified syndromes [Johns, 1995]. They involve the retina (pigmentary degeneration, KSS), the optic nerve (optic atrophy, Leber disease), the anterior chamber (cataract, corneal opacities), and also extra-ocular muscles (limitation of eye movements, ophthalmoplegia, diplopia, lid ptosis, in the progressive external ophthalmoplegia, and KSS).

### Dermatological Symptoms

Hair and skin anomalies include mottled pigmentation of photo-exposed areas, acrocyanosis, hypertrichosis, alopecia, and abnormal hairs [Rötig et al., 1992; Rötig et al., 1993; Bodemer et al., 1999]. Hair anomalies consist of dry, thick and brittle hair shafts. Amino acid analysis of the hair is largely normal. Electron microscopy shows transverse fractures across the hair shafts through the cuticle and hairs displaying twists (*pili torti*), longitudinal grooving, cuticle loss, and *trichorrhexis nodosa* [Bodemer et al., 1999].

Palmoplantar keratoderma affecting the plantar surfaces has been reported in association with deafness in large pedigrees segregating with the mitochondrial A7445G mutation [Seviour et al., 1998]. Epidermis biopsy revealed marked hyperkeratosis, increased thickness of the granular layer, and moderate acanthosis. Less frequent features include epidermal naevus, ichthyosis, and cholesteatoma. While interactions between mitochondria and cytokeratins are known to occur, the link between the A7445G mutation and palmoplantar keratoderma remains unclear.

### Facial Dysmorphism

Mitochondrial disorders are not commonly regarded as causes of malformations. Yet, RC deficiency has been observed in several patients with progressive facial anomalies, microcephaly and antenatal and postnatal growth retardation [Cormier-Daire et al., 1996]. Facial features include round face, high forehead, small nose, and long flat philtrum, reminiscent of fetal alcohol syndrome. Low-set, posteriorly angulated ears with deficient helix and hypoplastic lobules suggestive of a CHARGE (colobome-heart-choanal atresia-mental retardation-genito-urinary syndrome) association were occasionally observed. Limb and trunk involvements include short hands, brachydactyly, hypoplasia of distal and middle phalanges, hypoplastic nails, and the VATER (vertebral-anal-tracheo-oesophageal-renal syndrome) association [Cormier-Daire et al., 1996, Damian et al., 1996]. Some of these anomalies common to fetal alcohol syndrome and metabolic diseases have been described in pyruvate dehydrogenase (PDH) deficiency [Robinson et al., 1987]. It has been proposed that in fetal alcohol syndrome, a product of alcohol oxidation, acetaldehyde, inhibits the PDH complex and may be responsible for secondary PDH deficiency in utero. Whether facial dysmorphism in RC deficiency is related to an intoxication of the fetus by abnormal intermediates as well, is presently unknown.

### THE SYNDROMES OF RESPIRATORY CHAIN DEFICIENCY

Attempts to delineate tight boundaries between syndromes are questionable, especially as the nature, clinical course, and severity of recruited symptoms vary among (and even within) affected individuals. However, some specific clinical associations have been occasionally recognized as distinct syndromes. Molecular genetic studies have demonstrated that these associations are not fortuitous. The most frequent syndromic forms of RC deficiency are given in the alphabetic order.

Children meeting the criteria of Alpers progressive sclerosing poliody-strophy have RC deficiency in their liver. Children suffered myoclonus epilepsy, related to the gradual involvement of the gray matter, and developed delayed onset hepatic involvement frequently triggered by valproate intake. Liver enlargement with mild cytolysis and steatosis were noted and the children frequently died shortly thereafter [Cormier-Daire et al., 1997; DiMauro et al., 1990]. Plasma and CSF lactate was normal and expression of the enzyme deficiency was usually restricted to the liver. Considering the severe prognosis and the expected recurrence risk (25%), children with unexplained myoclonus epilepsy in early infancy should be screened for RC enzyme deficiency in a needle biopsy of their liver.

Barth syndrome is an X-linked dilated cardiomyopathy, with cyclic neutropenia and skeletal myopathy and abnormal mitochondria [Barth et al., 1983]. This condition has been mapped to chromosome Xq28 and ascribed to mutations of the tafazzin gene (G4.5) [Bolhuis et al., 1991; Bione et al., 1996]. This gene might also account for non-syndromic forms of X-linked cardiomyopathy and isolated non-compaction of the left ventricle myocardium [Bleyle et al., 1997].

Dominant optic atrophy is the most prevalent hereditary optic atrophy resulting in a progressive loss of visual acuity, centrocoecal scotoma, and bilateral temporal atrophy of the optic nerve. It has been ascribed to mutations in the OPA1 gene encoding a dynamin-related protein localized to mitochondria. Anomalies of mitochondrial integrity and distribution are suspected to result from impairment of the protein function [Alexander et al., 2000; Delettre et al., 2000].

Friedreich ataxia is a common autosomal recessive degenerative disease characterized by cerebellar ataxia with progressive gait and limb ataxia, dysarthria, lack of tendon reflexes and pyramidal weakness of the inferior limbs and hypertrophic cardiomyopathy [Dürr et al., 1996]. Friedreich ataxia is caused primarily by a GAA repeat expression in

the first intron of the frataxin gene on chromosome 9q13 [Campuzano et al., 1996]. The function of the protein is unknown but mutated frataxin triggers deficiency of iron-sulphur (Fe-S) cluster-containing enzymes, such as RC complexes I, II, and III and aconitase in heart (and possibly brain) of Friedreich ataxia patients [Rötig et al., 1997]. The deficiency of Fe-S dependent enzymes in Friedreich ataxia is presumably related to increased mitochondrial oxidative stress, since Fe-S proteins are remarkably sensitive to oxygen free radicals. Friedreich ataxia should be regarded, therefore, as a frequent nuclear encoded mitochondrial disorder [Rötig et al., 1997].

Hereditary paraganglioma is characterized by the development of benign vascularized tumors in the head and neck, with the most common tumor site found in the carotid body, a chemoreceptive organ sensing blood oxygen level. Mutations in the SDHD and SDHC genes, encoding the two anchoring subunits of the complex II (see above), have been recently reported to cause hereditary paraganglioma [Baysal et al., 2000; Nieman and Müller, 2000].

Hereditary spastic paraplegia is characterized by progressive weakness and spasticity of the lower limbs due to degeneration of the corticospinal axons. An autosomal recessive form has been mapped to chromosome 16q24.3. The gene, SPG7, encodes paraplegin, a protein that localizes to mitochondria and is highly homologous to the yeast metalloproteases with proteolytic and chaperon-like activities at the inner mitochondrial membrane. The exact function of the protein is unknown, but mutated paraplegin results in RC defects, suggesting that this form of hereditary spastic paraplegia should be regarded as a mitochondrial disorder [Casari et al., 1998].

Kearns-Sayre syndrome (KSS) is a multi-system disorder characterized by the invariant triad: onset before age 20, progressive external ophthalmoplegia, pigmentary retinal degeneration, plus at least one of the following: complete heart block, CSF protein above 100

mg/dl, cerebellar ataxia. Large-scale heteroplasmic mtDNA deletions are frequently detected in skeletal muscle (rarely in other tissues) [Lestienne and Ponsot, 1988; Moraes et al., 1989].

Leber hereditary optic neuroretinopathy (LHON) is associated with rapid bilateral central vision loss due to optic nerve death [Johns, 1995]. Cardiac dysrhythmia is frequently associated with the disease but no evidence of skeletal muscle pathology or gross structural mitochondrial abnormality has been documented. The median age of vision loss is 20 to 24 years, but it can occur at any age between adolescence and late adulthood. Expression among maternally-related individuals is variable and there is a bias toward males being affected. To date, the disease has been associated with three primary mutations in the mtDNA (that can act autonomously) and 15 secondary mutations (that may act in association with each other to cause the disease) [DiMauro and Schon, 2001].

Leigh subacute necrotizing encephalomyelopathy is a devastating encephalopathy characterized by recurrent attacks of psychomotor regression with pyramidal and extra pyramidal symptoms, leukodystrophy, and brainstem dysfunction [Valanne et al., 1998]. The pathological hallmark consists of focal, symmetrical, and necrotic lesions in the thalamus, brainstem, and the posterior columns of the spinal cord. Microscopically, these spongiform lesions show demyelination, vascular proliferation, and astrocytosis. The most common causes are deficiency of either RC complex I, or II, or IV, or of pyruvate dehydrogenase or the T8993G mtDNA mutation [Rahman et al., 1996; Kirby et al., 2000; Makino et al., 2000] in association with mtDNA or nDNA mutations [Tatuch et al., 1992; Kogelnik et al., 2000]. Nuclear gene mutations include mutations in the gene encoding the flavoprotein subunit of complex II [Bourgeron et al., 1995], in several CI-subunit encoding genes (NDUFV1, NDUFS8, NDUFS7) [Triepels et al., 2001] and in a COX assembly gene, SURF1 [Tiranti et al., 1995; Zhu et al., 1998].

Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) is characterized by onset in childhood with intermittent hemi-cranial headache, vomiting, proximal limb weakness, recurrent neurological deficit resembling strokes (hemiparesis, cortical blindness, hemi-anopsia), lactic acidosis, and occasionally ragged red fibers in the muscle biopsy [Valanne et al., 1998]. Computed tomography (CT) brain scan shows low-density areas (usually posterior) that may affect both white and gray matter but does not always correlate to clinical symptoms or vascular territories. The pathogenesis of stroke-like episodes in MELAS has been ascribed to either cerebral blood flow disruption or acute metabolic decompensation in biochemically deficient areas of the brain. The disease is most frequently caused by a mutation in the tRNA<sup>Leu</sup> gene (A3243G) [Goto et al., 1990; Kogelnik et al., 2000].

Mitochondrial myopathy, peripheral neuropathy, encephalopathy, and gastro intestinal disease (MNGIE) manifests as intermittent diarrhea and intestinal pseudo obstruction (myoneurogastro intestinal encephalopathy) [Hirano et al., 1994]. Mutations in the thymidine phosphorylase-encoding gene leading to multiple mtDNA deletion and anomalies in mtDNA synthesis have been reported in 12 patients [Nishino et al., 1999].

Myoclonus epilepsy with ragged red fibers (MERRF) is an encephalomyopathy with myoclonus, ataxia, hearing loss, muscle weakness, and generalized seizures [Wallace et al., 1988]. A missense mutation in the mt tRNA<sup>Lys</sup> gene (A8344G) accounts for 80% of MERRF cases [Shoffner et al., 1990; Kogelnik et al., 2000].

Neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP) and variable sensory neuropathy, seizures, and mental retardation is due to an amino-acid change in the ATPase6 gene (T8993G) [Holt et al., 1990; Kogelnik et al., 2000].

Pearson syndrome is a syndrome of refractory sideroblastic anemia, with variable neutropenia and thrombocyto-

penia, vacuolization of marrow precursors and exocrine pancreatic dysfunction. Half of the patients have anemia and half have diarrhea as the initial symptom, but all have an impaired redox status in plasma. Both sexes are affected. Severe transfusion-dependent macrocytic anemia begins in early infancy (before one year) and the disease is fatal before 3 years of age in 62% of cases [Rötig et al., 1990, Rötig et al., 1995b]. The patients who survive spontaneously recover from their myelodysplasia but usually develop KSS. Interestingly, several KSS patients whose disease apparently started in childhood or adulthood were retrospectively shown to have experienced transient sideroblastic anemia, neutropenia, chronic watery diarrhea, or failure to thrive of unexplained origin in early infancy [MacShane et al., 1991]. Large-scale heteroplasmic deletions/duplications of mtDNA are constantly observed in affected and non-affected organs. Directly repeated sequences are consistently found at the boundaries of the rearrangements, suggesting that these repeats might have triggered molecular mtDNA recombinations [Kogelnik et al., 2000].

Progressive external ophthalmoplegia (PEO) is a mitochondrial myopathy with progressive muscle weakness and external ophthalmoplegia. Ataxia, episodic ketoacidotic coma, and early death have been reported associated with single [Moraes et al., 1989] or multiple mtDNA deletions [Zeviani et al., 1989]. Mutations in the gene encoding the muscle isoform of the adenylate carrier (ANT1) mapping to chromosome 3p [Kaukonen et al., 1996] have been recently reported to be one cause of multiple mtDNA deletions in PEO, presumably due to abnormal nucleotide availability for mtDNA synthesis [Kaukonen et al., 2000].

Syndromic forms of sensorineural hearing loss are not exceptional. The diabetes mellitus and deafness association is frequently caused by the A3243G mutation in the tRNA<sup>Leu</sup> gene, also associated with MELAS [van den Ouweland et al., 1992]. Hearing loss usually develops after onset of diabetes. The deafness-ataxia-myoclonus syn-

drome is caused by a single nucleotide insertion in the tRNA<sup>Ser</sup> gene (C7472) [Tiranti et al., 1995]. The deafness-palmoplantar keratoderma association is a recently identified syndrome, ascribed to a maternally-inherited mutation altering the tRNA<sup>Ser</sup> stability [Seviour et al., 1998]. Indeed, the A7445G mutation is adjacent to the 3' end of the tRNA<sup>Ser</sup> on the light strand and a silent change in the stop codon of the COXI gene on the heavy strand.

Wolfram syndrome is a syndrome of diabetes mellitus and insipidus with optic atrophy and deafness (DIDMOAD). While most cases have been ascribed to an autosomal recessive gene mapping to chromosome 4p16, [Polymeropoulos, 1994] some cases of early-onset DIDMOAD might result from mtDNA mutations [Pilz et al., 1994] or deletions [Rötig et al., 1993].

## METABOLIC SCREENING IN VIVO

The current screening of RC deficiency includes the determination of plasma lactate, pyruvate, and ketone bodies and their molar ratios, as indexes of oxidation/reduction status in cytoplasm and mitochondria, respectively. Determinations should be made in the fasted and 1-hour fed individuals and repeated during the day. In order to avoid artefactual elevation of lactic acid, blood samples should be taken from a patient at rest through a heparinized venous catheter and immediately deproteinized by perchloric acid [Trijbels et al., 1993]. Samples should be either forwarded in ice to the laboratory or immediately frozen ( $-20^{\circ}\text{C}$  or below). Blood glucose and non-esterified fatty acids should be simultaneously monitored.

The observation of a persistent hyperlactatemia ( $> 2.5$  mM) with elevated L/P ( $> 20$ ) and ketone body molar ratios is highly suggestive of a RC deficiency (particularly in the post-absorptive period) [Robinson, 1995; Munnich et al., 1996a]. In addition, investigation of the redox status in plasma can help discriminate between the different forms of congenital lactic acidosis, based on L/P and ketone body

molar ratios in vivo. Indeed, an impairment of oxidative phosphorylation usually results in L/P ratios above 20 and ketone body ratios above 2, whereas

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### *Redox status in plasma can help discriminate between the different forms of congenital lactic acidosis.*

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a defect of the pyruvate dehydrogenase (PDH) complex results in low L/P ratios (below 10). When basal screening tests are inconclusive, a glucose-loading test (2 g/kg, orally) can unmask latent hyperlactatemia and/or paradoxical hyperketonemia [Touati et al., 1997]. The urinary excretion of Krebs cycle intermediates and/or 3-methyl-glutaconic aciduria has a good diagnostic value but is non-specific, as it is frequently encountered in a variety of RC enzyme deficiency syndromes [Gibson et al., 1993]. When the redox status in plasma is not altered, the determination of the redox status in the CSF can be performed. The amino acid chromatography shows indirect evidence of hyperlactatemia (i.e., elevated plasma  $\alpha$ -alanine and proline) and occasionally hypermethioninemia. Interestingly, plasma citrulline is often low in complex V deficiency caused by the NARP mutation. More generally, hypocitrullinemia might be a non-specific hallmark of impaired oxidative phosphorylation in vivo. In RC deficiency, the synthesis of circulating citrulline by enterocytes might be limited by the reduced availability of mitochondrial ATP, required for carbamyl phosphate synthase I activity.

Yet, pitfalls in the metabolic screening are numerous and the above diagnostic tests may fail to detect an impaired redox status in plasma for several reasons:

1. Proximal tubulopathy may lower blood lactate and increase urinary lactate;
2. Diabetes mellitus may hamper entry of pyruvate into the citric acid cycle;

3. Tissue-specific isoforms may be selectively impaired, barely altering the redox status in plasma;
4. The defect may be generalized but partial: The more those tissues with higher dependence on oxidative metabolism suffer (such as brain and muscle), the more the oxidation-reduction status is impaired in plasma.

When screening tests are negative, RC deficiency may be misdiagnosed. For this reason, the investigation of patients at risk of RC deficiency should include the systematic screening of all possible target organs and tissues regardless of the onset symptom, as multiple organ involvement is an important diagnostic clue in RC deficiency.

## DIAGNOSTIC TESTS

Diagnostic tests include spectrophotometric enzyme assays and functional studies of intact mitochondria, each providing an independent clue to diagnose RC deficiency. A variety of functional studies can be performed,

### *Enzyme assays and functional studies each providing an independent clue to diagnose RC deficiency.*

including oxidation of radioactive substrates, analysis of mitochondrial redox balance, ATP synthesis, and the widely used polarographic studies [Trijbels et al., 1997; Robinson, 1998; Kirby et al., 1999].

Polarographic studies consist of the measurement of oxygen consumption by cells or mitochondria-enriched fractions using a Clark electrode in the presence of various substrates (malate + pyruvate, malate + glutamate, succinate, palmitate, etc.) [Chretien et al., 1994; Rustin et al., 1994b]. In case of complex I deficiency, polarographic studies show impaired respiration with NADH-producing substrates, while respiration and phosphorylation are normal with

FADH<sub>2</sub>-producing substrates (succinate). The opposite is observed in case of complex II deficiency, whereas a block at the level of complexes III or IV impairs oxidation of both NADH- and FADH<sub>2</sub>-producing substrates. In complex V deficiency, there is an impaired oxidation with various substrates, but adding an uncoupling agent returns the oxidation rate to normal, suggesting that the limiting step involves phosphorylation rather than the RC. Polarographic studies may also detect PDH deficiency, citric acid cycle enzyme deficiency, and defects of coenzymes, carriers, and shuttles (including cytochrome *c*, ubiquinone, cations, and adenylate), as these conditions also impair the production of reducing equivalents in the mitochondrion. In these cases, however, independent enzyme activities are expected to be normal (see below).

The scaled-down procedures now available allow the rapid recovery of mitochondria-enriched fractions (400–500 µg proteins) from small skeletal muscle biopsies (100–200 mg obtained under local anesthesia) and make polarographic studies feasible in infants and children. Measurement of oxygen consumption by intact or detergent-permeabilized circulating lymphocytes (isolated from 10 ml of blood on a Percoll cushion) and cultured cells (lymphoblastoid cell lines, skin fibroblasts) is also feasible and represents a non-invasive and easily reproducible diagnostic test. The only limitation of these techniques is the absolute requirement of fresh material; no polarographic or other functional studies are possible on frozen material.

Spectrophotometric studies consist of isolated or combined RC enzyme assays, using specific electron donors and acceptors. They do not require isolation of mitochondrial fractions and can be carried out on tissue homogenates. For this reason, the amount of material required for enzyme assays (1–20 mg) is very small and can be easily derived from needle biopsies of liver, kidney, and endomyocardial biopsies or from a pellet of lymphocytes or cultured skin fibroblasts. Samples can be immediately

frozen and kept dry in liquid nitrogen (or at –80°C) [Taylor et al., 1993; Birch-Machin et al., 1994; Chretien et al., 1994; Rustin et al., 1994b; Chretien et al., 1998; Miro et al., 1998].

The question of what tissue should be investigated deserves particular attention. In principle, a relevant tissue is one that clinically expresses the disease. In case of muscle weakness, the appropriate working material is a skeletal muscle micro biopsy (deltoid). When the hematopoietic system expresses the disease (i.e., Pearson syndrome), tests should be carried out on circulating lymphocytes, polymorphonuclear cells, or bone marrow. In case of liver disease or cardiomyopathy, a needle biopsy of the liver [Cormier-Daire et al., 1997] or an endomyocardial biopsy [Rustin et al., 1994b] are usually feasible. When the disease is essentially expressed in a barely accessible organ (brain, retina, endocrine, smooth muscle), peripheral tissues should be extensively tested (including skeletal muscle, cultured skin fibroblasts, circulating lymphocytes). Whatever the affected organ, it is important to take skin biopsies from patients for subsequent investigations on cultured fibroblasts (even post mortem).

It should be borne in mind however that the *in vitro* investigation of oxidative phosphorylation remains difficult and several pitfalls should be put forward:

1. Normal RC enzyme activities can be found in an organ or tissue that does not clinically express the disease. One might be dealing with a tissue-specific organ deficiency, as observed in Friedreich Ataxia [Rötig et al., 1997].
2. Normal RC enzyme activity does not preclude mitochondrial dysfunction, even if the tissue tested clinically expresses the disease. One might be dealing with a kinetic mutant, tissue heterogeneity, or cellular mosaicism (heteroplasmy, see below). In this case, one should pay particular attention to histo-enzymatic investigations, carry out extensive molecular genetic analyses, test other tissues, and possibly repeat investigations.

3. The apparent discrepancy between normal complex I, II, and III activities and impaired combined complex I-III or II-III activities is indicative of a deficient quinone pool. Inborn errors of quinone synthesis remain largely unknown but worthwhile to recognize, as these rare forms of RC deficiency respond to quinone administration in vitro and in vivo [Ogasahara et al., 1989; Rötig et al., 2000]. On the other hand, incorrect freezing may result in a rapid loss of quinone-dependent activities, probably due to peroxidation of membrane lipids [Rustin et al., 1994a]. Tissue samples fixed for morphological studies are inadequate for RC enzyme assays.
4. The scattering of control activities and the frequent accumulation of partially-defective mitochondria in patients' cells occasionally hamper the recognition of enzyme deficiencies, as normal values frequently overlap those found in the patients. It is helpful to express results as ratios, especially as normal oxidative phosphorylation requires balanced ratios of RC enzyme activities. Using activity ratios, patients whose absolute activities are in the low normal range can be unambiguously diagnosed as enzyme deficient [Chretien et al., 1998]. Yet, this way of expressing results may fail to identify generalized defects.
5. No reliable method is presently available for the direct measurement of complex I activity in circulating or cultured cells, due to the rotenone-resistant cellular NADH-cytochrome *c* reductase activity. An indirect estimation of complex I activity can, however, be obtained by the study of NADH-dependent substrate oxidation or ATP synthesis [Robinson, 1998; Kirby et al., 1999].
6. The phenotypic expression of RC enzyme deficiencies caused by mtDNA mutations in cultured cells can be unstable and activities may tend to return to normal values when cells are grown in a standard medium. Adding uridine (200  $\mu$ M) and pyruvate (4 mM) to the culture medium

prevents a counter selection of RC deficient cells, thereby stabilizing the mutant phenotype (the availability of uridine, required for nucleic acid synthesis, is reduced by the secondary deficiency of the RC-dependent dihydroorotate dehydrogenase activity) [Gérard et al., 1992; Bourgeron et al., 1993].

7. Discrepancies between control values may indicate faulty experimental conditions. Relative activities should be consistent when tested under non-rate-limiting conditions. For example, normal succinate-cytochrome *c* reductase activity should be twice as high as normal succinate-quinone dichlorophenol indophenol reductase activity (because one  $e^-$  is required to reduce cytochrome *c* while two  $e^-$  are required to reduce dichlorophenol indophenol).

### Histopathological Studies

The muscle specimen taken under local anesthesia must be immediately frozen in liquid nitrogen-cooled isopentane. The histological hallmark of mitochondrial myopathy is the ragged red fiber (RRF) demonstrated with the modified Gomori trichrome stain, containing peripheral and inter-myofibrillar accumulations of abnormal mitochondria [Romero et al., 1996]. Although the diagnostic importance of RRF is undisputed, it is now clear that absence of RRF does not rule out the diagnosis of mitochondrial disorder, since RRF are found in only a small minority of children with RC defects. Various

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### ***RRF are found in only a small minority of children with RC defects.***

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histochemical stains specific for oxidative enzymes are used to analyze the distribution of mitochondria in individual fibers and assess enzymatic activities. Histochemical stains are particularly valuable for detecting heterogeneity of enzyme deficiency in a

given muscle section. Myofibril integrity and muscle type fiber predominance and distribution can be evaluated with the myofibril ATPase stain and studies using antibodies directed against specific subunits are routinely performed in reference centers.

### Magnetic Resonance Spectroscopy (MRS) of Muscle and Brain

Phosphorus MRS allows the study of muscle and brain energy metabolism in vivo. Inorganic phosphate (Pi), phosphocreatine, adenosine mono-, di-, or tri-phosphate (AMP, ADP, ATP), and intracellular pH may be measured. The Pi/phosphocreatine ratio is the most useful parameter and may be measured at rest, during exercise, and during recovery. An increased ratio is found in most patients and MRS is becoming a useful tool for both diagnosing mitochondrial diseases and monitoring therapeutic trials. Yet, the observed anomalies are not specific to RC enzyme deficiencies and no correlation between MRS findings and the RC defect can be made [Radda et al., 1995].

### Molecular Genetics Studies

#### *mtDNA mutations*

The genetic investigation of a mitochondrial disorder deserves an extensive pedigree reporting on minor signs in relatives. This information is of particular importance for deciding what molecular studies should be carried out first. For example, maternal inheritance points toward mtDNA mutations, while autosomal dominant inheritance points toward multiple mtDNA deletions. Sporadic cases and cases consistent with autosomal recessive inheritance (consanguineous parents) should be tested for mtDNA deletion-duplications and mtDNA depletion, respectively. The molecular genetic investigation of mtDNA is not a routine procedure and several pitfalls should be stressed:

1. A mixture of two populations of mtDNA molecules does not always indicate a mutation as it may be the result of mtDNA polymorphisms.

However, heteroplasmic polymorphisms are extremely rare, while homoplasmic polymorphisms are frequent in mtDNA and may constitute a more common problem encountered when studying RFLP patterns or sequencing mtDNA [Thorburn, 2000].

2. The distribution of mutated mtDNA molecules may differ widely among tissues (heteroplasmy), possibly accounting for variable clinical expression. The tissue to be investigated is the one that actually expresses the disease (mtDNA deletions are frequently absent in circulating lymphocytes of KSS patients).
3. mtDNA deletions may be occasionally associated with duplications. Due to the symmetry of several rearrangements, detection of duplication requires enzymatic cleavage of the mtDNA at sites located within the deletion [Ballinger et al., 1994; Poulton et al., 1994; Rötig et al., 1995].
4. mtDNA rearrangements are unstable and gradually disappear in cultured cells unless uridine is included in the culture medium. No conclusion can be drawn from the absence of an mtDNA mutation in cell cultures grown in standard conditions [Bourgeron et al., 1993].
5. The detection of mtDNA depletion requires systematic re-hybridization of Southern blots using a control nuclear DNA probe for densitometric determination of the mtDNA/nuclear DNA ratio [Moraes et al., 1991; Poulton and Holt, 1994].
6. Finally, while a positive test supports the mitochondrial nature of the disease, a negative result does not rule out an mtDNA mutation nor does it represent a clue that a nuclear mutation is involved.

### Nuclear DNA Mutations

The number of disease-causing mutations in nuclear genes is steadily growing, presumably underlying the vast majority of RC deficiencies. It is worth bearing in mind that mtDNA deletions

and mutations account for no more than 10%–15% of patients, at least among pediatric cases. Thus, in most cases, nuclear gene defects are presumably responsible for RC deficiency.

Disease-causing nuclear gene mutations fall in two categories: 1) mutations in nuclear-encoded RC subunits, and 2) mutations in assembly and maintenance protein genes. Surprisingly, while the chromosomal location and cDNA sequence of most RC subunit genes are known, only a small number of catalytic subunit gene mutations have been reported, namely a mutation in the gene for complex II flavoprotein [Bourgeron et al., 1995] and mutations in nuclear encoded complex I genes, including NDUFS2, NDUFS4, NDUFS7, NDUFS8, and NDUFV1 [Triepels et al., 2001] in Leigh syndrome. Yet, mutations are found in the most conserved nuclear encoded complex I subunit genes and account for only one third of patients with isolated complex I deficiency. Moreover, systematic sequence analysis of nuclear encoded subunits of complex IV has failed to detect any mutation in their coding sequences. Taken together, these data support the view that mitochondrial assembly and maintenance are primarily altered in RC deficiency. The discovery of deleterious mutations in the genes encoding four proteins that are essential for the proper assembly of functional complex IV, namely SURF1 [Tiranti et al., 1995; Zhu et al., 1998], heme A:farnesyl transferase (COX10) [Valnot et al., 2000b], SCO1 [Valnot et al., 2000a], and SCO2 [Papadopoulou et al., 1999] in Leigh disease, de Toni-Debré-Fanconi syndrome, hepatoencephalopathy, and cardioencephalomyopathy, respectively, gives strong support to this view. Along the same lines, it is worth remembering that two non-RC subunit genes, thymidine phosphorylase and tafazzin, account for MNGIE and Barth syndrome, respectively (see above). Hopefully, the clinical presentations and the biochemical defects associated with mutations in these genes might be shown to be sufficiently homogenous as to allow a targeted screening for mutation analyses. Thus, the recognition of

COX deficiency in a patient presenting with Leigh syndrome should prompt the screening for mutations in the SURF1 gene. Yet SURF1 mutations can cause villous atrophy, growth rate retardation, and early death, without evidence for Leigh syndrome that perhaps may have developed later [von Kleist-Retzow et al., 2001].

Determining genotype/phenotype relationships for nuclear-encoded mitochondrial disorders is complicated by a number of factors, including: 1) several hundred nuclear genes may be involved, 2) functional similarity does not provide any clue, e.g., mutations in COX assembly genes, SURF1 [Zhu et al., 1998], COX10 [Valnot et al., 2000a], SCO1 [Valnot et al., 2000b], and SCO2 [Papadopoulou et al., 1999] all result in distinct clinical presentations, 3) unpredictable functional consequences may arise from mutations in some of these genes, e.g., mtDNA multiple deletions resulting from mutations in ANT1 gene. Thus, elucidating the genetic bases of RC deficiency of nuclear origin promises to be a difficult task, which will have to take advantage of new strategies and technologies yet to be defined.

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