Short report

A mitochondrial encephalomyopathy with a partial cytochrome *c* oxidase deficiency of muscle

P M M VAN ERVEN, F J M GABREËLS, W RUITENBEEK, W O RENIER, H J TER LAAK, A M STADHOUDERS

From the Institutes of Neurology, Pediatrics and Submicroscopic Morphology, University Hospital Nijmegen, The Netherlands

SUMMARY A 16 year old girl showed delayed psychomotor development. In infancy, exercise intolerance, cerebellar signs, deteriorated with increasing intercurrent infections, and disturbances of breathing and cardiac rhythm became manifest. From the age of 7 years there was chronic progressive psychomotor deterioration, with hypotonia, a bilateral pyramidal and cerebellar syndrome, and mild epilepsy. CSF pyruvate and lactate levels were elevated, and lactate content was elevated in the urine. There was an abnormally high rise of lactate levels on moderate exercise and an abnormal response to pyruvate loading. Quadriceps muscle biopsies obtained at age 10 and 16 years showed ragged-red fibres, and a decreased cytochrome *c* oxidase activity and cytochrome *aa₃* content. Cytochrome *c* oxidase activity in fibroblasts was normal. Clinical signs and symptoms in association with a disturbance of mitochondrial energy metabolism led us to a diagnosis of probable Leigh syndrome.

In the clinical spectrum of mitochondrial myopathies Petty *et al*¹ identified three subgroups: (1) chronic progressive external ophthalmoplegia (CPEO) and limb weakness, (2) proximal weakness with fatigability, (3) predominantly or exclusively central nervous system (CNS) disease. The recognition that CNS disease is a prominent feature in a subgroup of the mitochondrial myopathies prompted Shapira *et al*² to expand the concept of mitochondrial myopathy to mitochondrial encephalomyopathy. This latter group of disorders encompasses the syndromes of Alpers,³ Leigh,⁴ dysmyelination,⁵ myoclonus epilepsy associated with ragged-red fibres (MERRF)⁶ and a syndrome indicated by the acronym MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes).⁷

We present a girl with a mitochondrial encephalomyopathy and a clinical diagnosis of probable Leigh syndrome and a partial cytochrome *c* oxidase deficiency of muscle.

Case reports

The patient

The patient is the youngest child of healthy non-consanguineous parents. There is one healthy sib. The oldest sib died at age 12 after an accident. Family history reveals no neurodegenerative disorders. Two sibs of the father suffer from epilepsy. Pre-, peri- and postnatal periods of the patient were uneventful. Psychomotor development was delayed. She was able to sit without help at age 18 months and walk at age 3 years. She spoke her first words at age 2½ years. Motor performance was poor and deteriorations often with slow recovery was noted with intercurrent infections. During these periods of deterioration, frequent sighing, tachy- and bradypnoea, and tachy- and bradycardia were evident. Occasionally she had partial oculomotor nerve paresis on the left, that subsided within one week. Progressive exercise intolerance became evident. At age 5 years, neurological examination revealed myopathic and cerebellar signs, brisk tendon reflexes and flexor plantar responses. Total IQ (WISC-R) was 95. At age 7 years, a slowly progressive psychomotor deterioration set in, and

Address for reprint requests: Dr Gabreëls, Institute of Neurology, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Received 23 June 1987 and in revised form 2 December 1987.
Accepted 7 December 1987

absence-like epileptic manifestations appeared, that were treated with ethosuccimide.

At age 10 years, she was referred to our department. Examination revealed a mentally retarded girl, with normal height, weight and skull circumference. There were no degenerative stigmata. General physical examination was normal. There was generalised hypotonia, muscle wasting, and proximal muscular weakness, with increased lumbar lordosis, genu recurvatum and a positive Gowers' sign. Facial expression was poor with mild bilateral ptosis, and monotonous dysarthric speech. Cerebellar involvement was shown by ataxia of rump and extremities, and marked dysidiadochokinesis. Tendon reflexes were brisk with spontaneous ankle clonus and bilateral Babinski signs. Steady slowly progressive deterioration of the neurological status occurred in the following years. She is now 16 years old with total IQ 53. She has been free of seizures and without anti-epileptic medication for the last 4 years.

Electrophysiological and radiological investigations

Electroencephalogram showed focal (right temporal) and generalised epileptic discharges. Electromyography and nerve conduction velocities were normal. Somatosensory, brainstem auditory and visual evoked potentials were within the normal ranges. Radiographs of skull, chest, hand and vertebral column, cerebral computed tomography and cerebral nuclear magnetic resonance imaging were normal. The electrocardiogram was normal and the echocardiogram showed no signs of cardiomyopathy.

Laboratory investigations

Standard blood, urinary and CSF studies⁸ revealed no abnormality. Serum levels of glucose, pyruvate, lactate, β -hydroxybutyrate and acetoacetate, their ratios, and responses of glucose, pyruvate and lactate to oral glucose

loading (1.75 g/kg) were in the normal ranges. Lactate excretion was elevated in one 24-hour urine sample (131 pmol/mmol creatine, normal <100). The McArdle/Fischbein ischaemic exercise test⁹ yielded a normal rise of serum lactate and ammonia levels. Moderate exercise (walking for a few minutes) gave a rise of serum pyruvate to 335 μ mol/l (normal 60–155 μ mol/l under resting conditions) and of serum lactate to 6480 μ mol/l (normal 460–1720 μ mol/l under resting conditions); the lactate-pyruvate ratio was 18.2 (normal up to 15) and serum pH remained normal with a base excess of -3.9 . An intravenous pyruvate loading test,^{10,11} (500 mg/kg) showed an abnormal response in that there was a rise of the lactate level after 5 minutes post-infusion to a maximum at 15 minutes.

In the CSF, the levels of glucose, β -hydroxybutyrate and acetoacetate were normal. The levels of pyruvate (222 μ mol/l, normal 85–132 μ mol/l) and lactate (1900 μ mol/l, normal 1200–1600 μ mol/l) were elevated with a normal lactate-pyruvate ratio.

Histopathological studies

Quadriceps muscle biopsies were performed at the age of 10 and 16 years. Histopathological studies showed no abnormalities except for the occurrence of ragged red fibres in both specimens. The cytochrome c oxidase stain showed a normal appearance in most fibres, but the ragged-red fibres showed strongly increased activity (fig. a). Electron-microscopy revealed no structural abnormalities of the mitochondria, and crystal-like inclusions did not occur. Cytochrome c oxidase activity was normal in all fibres investigated (fig. b).

Biochemical studies

In fresh homogenate of quadriceps muscle obtained by biopsy at age 10 years, pyruvate oxidation rate and activities

Fig Cytochrome c oxidase stain. The fibres show a normal activity except for the ragged-red fibre in the middle, that shows a strong increase of activity (bar = 50 μ m). (b) Electronmicrograph of section stained for cytochrome c oxidase activity. Normal activity is seen in all mitochondria ($\times 42,000$).

Table Results of biochemical studies in the 600 g supernatant of the two muscle tissue specimens

	Patient		Controls		
	1980	1986	Range	Mean, SD	n
Oxidation					
[1- ¹⁴ C]pyruvate + malate	208*		273-705	473, 117	20
[1- ¹⁴ C]pyruvate + carnitine	237*		266-941	504, 169	20
[U- ¹⁴ C]malate + pyruvate + malonate	270*		320-996	621, 211	20
[U- ¹⁴ C]malate + acetylcarnitine + malonate	343*		317-1155	575, 220	20
[U- ¹⁴ C]malate + acetylcarnitine + arsenite	196*		198-517	294, 80	19
ATP metabolism					
ATP + CrP production from pyruvate	2075†		3354-9993	5910, 2168	20
ATP + CrP production/pyruvate oxidation	10.0		8.8-15.0	11.8, 1.8	20
Enzyme activities					
Citrate synthase		41‡	48-146	77, 33	18
Cytochrome <i>c</i> oxidase	36‡	41	73-284	194, 92	39
Succinate:cytochrome oxidoreductase		8.7§	10-33	18, 7	9
NADH:O ₂ oxidoreductase		15.3	4.5-23	13, 7	7

*nmol ¹⁴CO₂/hr/mg protein; †nmol/hr/mg protein; ‡mU/mg protein; §nmol cytochrome *c* reduced/min/mg protein; ||nmol NADH oxidized/min/mg protein.

of citric acid cycle and respiratory chain were evaluated by measuring ¹⁴CO₂ production from [1-¹⁴C]pyruvate and [U-¹⁴C]malate.¹² ATP production by muscle homogenate was measured according to Ruitenbeek *et al.*¹³ The activity of cytochrome *c* oxidase¹⁴ was measured in muscle homogenate and in isolated mitochondria. Cytochrome content was measured in isolated mitochondria.¹⁵ Protein was assayed according to Lowry *et al.*¹⁶

At age 16 years a quadriceps muscle specimen was obtained by needle biopsy. Carnitine content,¹⁷ and activities of cytochrome *c* oxidase,¹⁴ citrate synthase,¹⁸ succinate-cytochrome *c* oxidoreductase¹⁹ and NADH:O₂ oxidoreductase²⁰ were measured, after storage of the muscle at -70 °C.

Oxidative metabolism was also evaluated in cultured fibroblasts by measuring ¹⁴CO₂ production rate from [1-¹⁴C]pyruvate and [2-¹⁴C]pyruvate,²¹ and the activities of cytochrome *c* oxidase¹² and citrate synthase.¹⁷

Results

The table shows the biochemical findings in both muscle specimens. ¹⁴CO₂ production rates from [1-¹⁴C]pyruvate and [U-¹⁴C]malate by muscle homogenate were slightly diminished. ATP + CrP production with pyruvate as substrate was also decreased. The ratio of the ATP + CrP production to the arsenite sensitive pyruvate oxidation was normal. Total carnitine content (carnitine + acylcarnitines) was normal in muscle homogenate. The specific activity of cytochrome *c* oxidase in muscle homogenate and in isolated mitochondria was about 20% of the mean control value. This enzymatic defect is associated with a lack of cytochrome *aa*₃. The ratio of the content of this protein of the cytochrome *c* oxidase complex to the content of cytochrome *b* and *c* + *c*₁ is diminished (0.59:1:1.20; control ratio 1.08:1:1.52). ¹⁴CO₂ production rates from

(1-¹⁴C]pyruvate and [2-¹⁴C]pyruvate by fibroblasts, and the activities of cytochrome *c* oxidase and citrate synthase were normal.

Discussion

We describe a 16-year-old girl with a slowly progressive degenerative neurological disorder characterised by myopathy, mental retardation, pyramidal, cerebellar and ocular signs, Frequent sighing, marked fluctuations of respiratory and cardiac rate, and long-lasting deteriorations with intercurrent infections are other clinical characteristics.

Laboratory investigations showed an elevation of lactate excretion in 24-hour urine. Lactate concentrations showed an abnormal increase on moderate exercise, and after pyruvate loading. In the CSF, levels of pyruvate and lactate were elevated. These findings are suggestive of a defect in pyruvate metabolism in this patient.

At the age of 10 and 16 years quadriceps muscle biopsy specimens showed ragged-red fibres. Biochemical studies showed a slight decrease of ¹⁴CO₂ production from pyruvate and malate, and a decrease of ATP production from pyruvate to one-third of the mean control value. Cytochrome *c* oxidase activity in muscle homogenate as well as in isolated muscle mitochondria was decreased to 20% of the mean control value. These data and the lack of cytochrome *aa*₃ protein point to a disturbance of mitochondrial oxidative metabolism at the level of cytochrome *c* oxidase. Involvement of the central nervous system and elevation of pyruvate and lactate in CSF make it likely that the same defect was present in CNS. Although the disorder had a multisystem character, no defect could be detected in fibroblasts.

In various patients with partial or complete cytochrome *c* oxidase deficiency, an overall decrease²²⁻²⁴ or complete lack^{25,26} of histochemical reactivity of cytochrome *c* oxidase have been reported. Apparently the residual activity of cytochrome *c* oxidase in our patient was sufficient to give normal light- and electronmicroscopic appearance.

Cytochrome *c* oxidase deficiency clinically presents in a heterogeneous fashion: it can manifest as a fatal infantile myopathy²⁷ with²⁸⁻³⁵ or without^{25,36-39} DeToni-Fanconi-Debré syndrome or as a benign spontaneously remitting myopathy of infancy.^{22,40} Cytochrome *c* oxidase deficiency has also been reported in two types of mitochondrial encephalomyopathies: the syndromes of Alpers⁴¹ and Leigh.^{23,26,40,42,43} Complete lack of histochemically demonstrable cytochrome *c* oxidase activity has been reported in individual fibres in patients with chronic progressive ophthalmoplegia, but biochemical studies most often revealed normal cytochrome *c* oxidase activity.^{44,45} The deficiency of cytochrome *c* oxidase described in Menkes' kinky hair disease⁴⁶ has a secondary nature, due to secondary deficiencies of copper dependent enzymes, one of which is cytochrome *c* oxidase.

Our patient fits in with the clinical picture of Leigh syndrome⁴⁷ and we have made a clinical diagnosis of Leigh syndrome in our patient.

Recently, a patient with a childhood encephalomyopathy with cytochrome *c* oxidase deficiency in muscle and platelets, resembling our patient, was reported by Angelini *et al.*²⁴ This patient, an 8 year old boy, had muscle wasting with proximal weakness, ataxia and mental impairment, but no pyramidal signs. There was parental consanguinity in this case, pointing to an autosomal recessive mode of inheritance.

In other patients with Leigh syndrome, the deficiency of cytochrome *c* oxidase was present in skeletal muscle, heart muscle, brain, kidney, liver and cultured fibroblasts.⁴⁸ Normal enzyme activities have been reported in liver tissue,²⁶ and in liver tissue and cultured fibroblasts⁴⁸ of Leigh patients with a cytochrome *c* oxidase deficiency of muscle.

There are strong indications of the existence of tissue-specific isoforms of the complex enzyme cytochrome *c* oxidase,⁴⁹ that might explain the differential involvement of tissues. The apoprotein consists of multiple subunits, three of which are encoded by mitochondrial DNA, whereas the rest is encoded by nuclear DNA.⁵⁰ As mitochondrial DNA is maternally transmitted,⁵¹ a non-mendelian type of inheritance is possible in cases of cytochrome *c* oxidase deficiency.⁵² However, this has until now not been documented in respiratory chain defects, and autosomal recessive inheritance seems most probable.

To elucidate aetiological mechanisms, enzyme abnormalities will have to be identified at the molecular level with modern techniques of immunocytochemistry and molecular genetics. Immunodetection allows identification of the enzyme subunit composition. The amount of immunologically reacting protein was normal in two Leigh patients with cytochrome *c* oxidase deficiency,^{42,53} and diminished³⁹ or absent⁵³ in patients with a fatal myopathy with or without renal dysfunction. As human mitochondrial DNA has been fully sequenced,⁵⁴ it is likely that mutations can be demonstrated at DNA level within a few years. Definition of biochemical errors at the molecular level will disclose different molecular defects of cytochrome *c* oxidase that constitute the basis of the different phenotypic expressions of the enzyme deficiency.

This investigation is part of the research program "Disorders of the Neuromuscular System" of the University of Nijmegen.

References

- Petty RKH, Harding AE, Morgan-Hughes JA. The clinical features of mitochondrial myopathy. *Brain* 1986;**109**:915-38.
- Shapira Y, Harel S, Russell A. Mitochondrial encephalomyopathies: a group of neuromuscular disorders with defects in oxidative metabolism. *Isr J Med Sci* 1977;**13**:161-4.
- Gabreëls FJM, Prick MJJ, Trijbels JMF, *et al.* Defects in citric acid cycle and the electron transport chain in progressive poliodystrophy. *Acta Neurol Scand* 1984;**70**:145-54.
- Van Erven PMM, Gabreëls FJM, Ruitenbeek W, *et al.* Subacute necrotizing encephalomyelopathy (Leigh syndrome) associated with disturbed oxidation of pyruvate, malate and 2-oxoglutarate in muscle and liver. *Acta Neurol Scand* 1985;**72**:36-42.
- Sengers RCA, Stadhouders AM, Trijbels JMF. Mitochondrial myopathies: clinical, morphological and biochemical aspects. *Eur J Pediatr* 1984;**141**:192-207.
- Fukuhara N, Tohiguchi S, Skirakawa K, Tsubahi T. Myoclonus epilepsy associated with ragged-red fibres (mitochondrial abnormalities): disease entity or a syndrome? Light- and electronmicroscopic studies of 2 cases and a review of the literature. *J Neurol Sci* 1980;**47**:117-33.
- Pavakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes: a distinctive clinical syndrome. *Ann Neurol* 1984;**16**:481-8.
- Van Erven PMM, Gabreëls FJM, Ruitenbeek W, Renier WO, Fischer JC. Mitochondrial encephalomyopathy: association with an NADH dehydrogenase deficiency. *Arch Neurol* 1987;**44**:775-8.
- Sinkeler SPT, Daanen HAM, Wevers RA, *et al.* The relation between blood lactate and ammonia in ischemic handgrip exercise. *Muscle Nerve* 1985;**8**:523-7.
- Dijkstra U, Gabreëls F, Joosten E, *et al.* Friedreich's ataxia: intravenous pyruvate load to demonstrate a defect in pyruvate metabolism. *Neurology* 1984;**34**:1493-7.
- Van Erven PMM, Gabreëls FJM, Wevers RA, *et al.* Intravenous pyruvate loading test in Leigh syndrome. *J Neurol Sci* 1987;**77**:217-27.
- Bookelman H, Trijbels JMF, Sengers RCA, Janssen AJM, Veerkamp JH, Stadhouders AM. Pyruvate oxidation in rat and

- human skeletal muscle mitochondria. *Biochem Med* 1978;20:395-403.
- 13 Ruitenbeek W, Sengers RCA, Trijbels JMF, Stadhouders AM, Janssen AJM. Estimation of energy metabolism in human skeletal muscle homogenate as a diagnostic aid. *J Inherited Metab Dis* 1981;4:91-2.
 - 14 Cooperstein SJ, Lazarow A. Microspectrophotometric method for determination of cytochrome oxidase. *J Biol Chem* 1951;189:665-70.
 - 15 Bookelman H, Trijbels JMF, Sengers RCA, Janssen AJM. Measurement of cytochromes in human skeletal muscle mitochondria, isolated from fresh and frozen stored muscle specimens. *Biochem Med* 1978;19:366-73.
 - 16 Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
 - 17 Parvin R, Pande SV. Microdetermination of (-) carnitine and carnitine acetyltransferase activity. *Anal Biochem* 1977;79:190-201.
 - 18 Srere PA. Citrate synthase. In: Löwenstein JM, ed. *Methods in Enzymology*, Vol XIII. London: Academic Press 1969:3-11.
 - 19 Fischer JC, Ruitenbeek W, Berden JA, et al. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. *Clin Chim Acta* 1985;153:23-6.
 - 20 Fischer JC, Ruitenbeek W, Trijbels JMF, et al. Estimation of NADH oxidation in human skeletal muscle mitochondria. *Clin Chim Acta* 1986;155:263-74.
 - 21 Willems HL, De Kort TFM, Trijbels FJM, Monnens LAH, Veerkamp JH. Determination of pyruvate oxidation rate and citric acid cycle activity in intact human leukocytes and fibroblasts. *Clin Chem* 1978;24:200-3.
 - 22 DiMauro S, Nicholson JF, Hays AP, et al. Benign infantile mitochondrial myopathy due to reversible cytochrome c oxidase deficiency. *Ann Neurol* 1983;14:226-34.
 - 23 Miyabayashi S, Narisawa K, Tada K, Sakai K, Kobayashi K, Kobayashi Y. Two siblings with cytochrome c oxidase deficiency. *J Inherited Metab Dis* 1983;6:121-2.
 - 24 Angelini C, Bresolin N, Pegolo G, et al. Childhood encephalomyopathy with cytochrome c oxidase deficiency, ataxia, muscle wasting, and mental impairment. *Neurology* 1986;36:1048-52.
 - 25 Boustany RN, Aprille JR, Halperin J, Levy H, DeLong GR. Mitochondrial cytochrome deficiency presenting as a myopathy with hypotonia, external ophthalmoplegia, and lactic acidosis in an infant and as fatal hepatopathy in a second cousin. *Ann Neurol* 1983;14:462-70.
 - 26 Willems JL, Monnens LAH, Trijbels JMF, et al. Leigh's encephalomyelopathy in a patient with cytochrome c oxidase deficiency in muscle tissue. *Pediatrics* 1977;60:850-7.
 - 27 DiMauro S, Hays AP, Eastwood AB. Different clinical expressions of cytochrome c oxidase deficiency. In: Scarlato G, Cerri C, eds. *Mitochondrial Pathology in Muscle Diseases*. Padua: Piccin Medical Books 1983:112-29.
 - 28 Van Biervliet JPM, Bruinvis L, Ketting D, et al. Hereditary mitochondrial myopathy with lactic acidemia, a DeToni-Franconi-Debré syndrome, and a defective respiratory chain in voluntary striated muscles. *Pediatr Res* 1977;11:1088-93.
 - 29 DiMauro S, Mendell JR, Sahenk Z, et al. Fatal infantile mitochondrial myopathy and renal dysfunction due to cytochrome-c-oxidase deficiency. *Neurology* 1980;30:795-804.
 - 30 Heiman-Patterson TD, Bonilla E, DiMauro S, Foreman J, Schotland DL. Cytochrome-c-oxidase deficiency in a floppy infant. *Neurology* 1982;32:898-900.
 - 31 Stansbie D, Dormer RL, Hughes IA, et al. Mitochondrial myopathy with skeletal muscle cytochrome oxidase deficiency. *J Inherited Metab Dis* 1982;5(Suppl 1):27-8.
 - 32 Minchom PE, Dormer RL, Hughes IA, et al. Fatal infantile mitochondrial myopathy due to cytochrome c oxidase deficiency. *J Neurol Sci* 1983;60:453-63.
 - 33 Müller-Höcker J, Pongratz D, Deufel T, Trijbels JMF, Endres W, Hübner G. Fatal lipid storage myopathy with deficiency of cytochrome c oxidase and carnitine. *Virchows Arch (Physiol)* 1983;399:11-23.
 - 34 Ohtani Y, Nishiyama S, Matsuda I. Renal handling of free and acylcarnitine in secondary carnitine deficiency. *Neurology* 1984;34:977-9.
 - 35 Zeviani M, Nonaka I, Bonilla E, et al. Fatal infantile mitochondrial myopathy and renal dysfunction caused by cytochrome c oxidase deficiency: immunological studies in a new patient. *Ann Neurol* 1985;17:414-7.
 - 36 Rimoldi M, Bottacchi E, Rossi L, Cornelio F, Uziel G, DiDonato S. Cytochrome c oxidase deficiency in muscles of a floppy infant without mitochondrial myopathy. *J Neurol* 1982;227:201-7.
 - 37 Trijbels F, Sengers R, Monnens L, et al. A patient with lactic acidemia and cytochrome oxidase deficiency. *J Inherited Metab Dis* 1983;6(Suppl 2):127-8.
 - 38 Sengers RCA, Trijbels JMF, Bakkeren JAJM, et al. Deficiency of cytochrome b and aa₃ in muscle from a floppy infant with cytochrome oxidase deficiency. *Eur J Pediatr* 1984;141:178-80.
 - 39 Bresolin N, Zeviani M, Bonilla E, et al. Fatal infantile cytochrome c oxidase deficiency: decrease of immunologically detectable enzyme in muscle. *Neurology* 1985;35:802-12.
 - 40 DiMauro S, Zeviani M, Servidei S, et al. Cytochrome oxidase deficiency: clinical and biochemical heterogeneity. *Ann NY Acad Sci* 1986;488:19-32.
 - 41 Prick MJJ, Gabreëls FJM, Trijbels JMF, et al. Progressive poliomyelopathy (Alpers' disease) with a defect in cytochrome aa₃ in muscle: a report of two unrelated patients. *Clin Neurol Neurosurg* 1983;85:57-70.
 - 42 Hoganson GE, Paulson DJ, Chun R, Sufit RL, Shug AL. Deficiency of muscle cytochrome c oxidase (CO) in Leigh's disease. *Pediatr Res* 1984;18:222A.
 - 43 Miyabayashi S, Narisawa K, Iinuma K, et al. Cytochrome c oxidase deficiency in two siblings with Leigh encephalomyelopathy. *Brain Dev* 1984;6:362-72.
 - 44 Johnson MA, Turnbull DM, Dick DJ, Sherratt HSA. A partial deficiency of cytochrome c oxidase in chronic progressive external ophthalmoplegia. *J Neurol Sci* 1983;60:31-53.
 - 45 Müller-Höcker J, Pongratz D, Hübner G. Focal deficiency of cytochrome c oxidase in skeletal muscle of patients with progressive external ophthalmoplegia. *Virchows Arch (Physiol)* 1983;402:61-71.
 - 46 Maehara M, Ogasawara N, Mizutani N, Watanabe K, Suzuki S. Cytochrome c oxidase deficiency in Menkes' kinky hair disease. *Brain Dev* 1983;5:533-40.
 - 47 Jellinger K, Seitelberger F. Subacute necrotizing encephalomyelopathy (Leigh). *Ergeb Inn Med Kinderheilkd* 1970;29:155-219.
 - 48 Arts WFM, Scholte HR, Loonen MCB, et al. Cytochrome c oxidase deficiency in subacute necrotizing encephalomyelopathy. *J Neurol Sci* 1987;77:103-15.
 - 49 Kadenbach B, Hartman R, Glanville R, Buse G. Tissue-specific genes code for polypeptide VIa of bovine liver and heart cytochrome c oxidase. *FEBS Lett* 1982;138:236-8.
 - 50 Tzagoloff A. *Mitochondria*. New York: Plenum Press 1982.
 - 51 Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. *Proc Natl Acad Sci USA* 1980;77:6715-9.
 - 52 Egger J, Wilson J. Mitochondrial inheritance in a mitochondrially mediated disease. *N Engl J Med* 1983;309:142-6.
 - 53 DiMauro S, Zeviani M, Bonilla E, et al. Cytochrome c oxidase deficiency. *Biochem Soc Trans* 1985;13:651-3.
 - 54 Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. *Nature* 1981;290:457-65.