MITOCHONDRIAL ENCEPHALOMYOPATHIES

AN ANALYSIS OF

CLINICAL AND LABORATORY DATA

OF PATIENTS AT THE RED CROSS

CHILDREN'S HOSPITAL.

INVESTIGATOR:

Dr GTM RIORDAN

M.B.Ch.B. F.C.P. (S.A.) Paed.

SUPERVISOR:

Professor PM LEARY

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DECLARATION

I,...GILLIAN RIORDAN..., hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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INTRODUCTION

The term "mitochondrial encephalomyopathy" encompasses a broad spectrum of clinical pathology related to mitochondrial dysfunction. In this review a brief historical account of developments in the field will be given, concentrating on the clinical aspects. A detailed account of advances in the understanding of mitochondrial molecular biology is beyond the scope of this text and readers are referred to authoritative reviews (1).

Mitochondrial disorders have gained increasing importance in clinical medicine in recent years. They not only deserve consideration in the differential diagnosis of many neuromuscular problems, but are known to be responsible for certain forms of cardiovascular, renal and hepatic disease, and can be linked to diabetes mellitus in some families. The role of mitochondrial dysfunction in the aging process is currently being explored.

The diagnosis is often overlooked as there are few specific clinical and biochemical tests accessible to the average clinician. The incidence of these disorders remains unknown and they may be far more common than originally thought.

The fundamental defect underlying mitochondrial disorders is an inadequate production of ATP to meet cellular energy demands. This may be masked by the presence of heteroplasmy, the coexistence of normal and mutated mitochondrial DNA (mtDNA) within a single cell. Thus the condition may remain occult unless subjected to stress in the form of exercise, carbohydrate loading or infection. It is probable that many carriers with minor defects remain asymptomatic, even under these conditions.

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MITOCHONDRIA

Mitochondria are intracellular organelles responsible primarily for the efficient production of adenosine triphosphate (ATP) to meet cellular energy demands. Although mitochondrial deoxyribonucleic acid (mtDNA) was discovered over thirty years ago (2), its relevance to human pathology has only recently been appreciated with the advent of new molecular techniques.

Mitochondrial DNA is unique in that it is inherited exclusively from the oocyte, resulting in maternal transmission of pathogenic mutations.

Structurally, mitochondrial DNA is a small, circular double-stranded molecule of 16,5 kilobase (kb). Its genetic code differs from the nuclear code, which has implications for future gene therapy. This reflects the probable bacterial origins of mitochondria. The genome has been entirely sequenced and codes for 13 proteins, two ribosomal ribonucleic acids (rRNAs) and 22 transfer RNAs (tRNAs). The protein genes all code for subunits of the respiratory chain: 7 subunits of nicotinamide adenine dinucleotide(reduced) dehydrogenase (NADH), 3 of cytochrome c oxidase, 2 of ATPase and 1 of cytochrome b.

There are on average between 2 and 10 copies of the mitochondrial genome in each mitochondrion and many hundred mitochondria in each cell. Thus each cell has thousands of copies of the mitochondrial genome. Normally these are identical in every cell, a situation known as homoplasmy. If a mutation exists it may be homoplasmic and involve all genomes, or, more commonly, may coexist with normal wild type DNA in a state of heteroplasmy. The degree of heteroplasmy may vary from tissue to tissue as a result of random distribution of mitochondria during cell division and possibly also as a consequence of a selective advantage of one type over another within tissues.

This random distribution explains the phenotypic variance which occurs within individuals expressing the same mutation. A pathogenic mutation will become clinically expressed only if present in sufficient proportion to wild type mtDNA to reduce nett energy metabolism within a particular tissue. This phenomenon is known as the threshold effect. Each tissue varies in susceptibility depending on its metabolic demands. As little as 10 percent of wild type mtDNA may offer protection from cellular dysfunction.

With the recent discovery of many disorders caused by mtDNA mutations, it has sometimes been dubbed the "25th chromosome". Despite this increase in knowledge, many questions remain unanswered.

Explanations for the high mutation rate in mtDNA have been its close proximity to free radicals generated by oxidative phosphorylation, its lack of protective histones and its limited DNA repair mechanisms (3). Deleted mtDNA may have a replicative advantage owing to its smaller size.

MtDNA deletions are responsible for the Kearns Sayre Syndrome (KSS): the other two commonly identified clinical syndromes, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) and myoclonus, epilepsy and ragged-red fibres, (MERRF), are caused by point mutations. The point mutations in both cases affect transfer RNA genes. A single base pair is substituted. In MERRF the transition is at base pair (bp) 8344 where guanine is substituted for adenine (A8344G) in the tRNA lysine gene. In MELAS there is a similar substitution at bp 3243 in the tRNA leucine gene. Point mutations in tRNA can cause multiple defects depending on the significance of the substitution on the function of the encoded protein.

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MITOCHONDRIAL ENCEPHALOMYOPATHIES:

A REVIEW OF THE LITERATURE

HISTORICAL PERSPECTIVE

Progress in the identification of clinical syndromes caused by mitochondrial mutations has gained increasing momentum in recent years. Stimulus to research in this direction started in 1959 with the publication of an article in Nature by Ernster, Ikkos and Luft describing the usefulness in clinical research of studies of mitochondrial enzyme activities (4). This was followed in 1962 by Luft's report (5) of a 35 year old woman with severe non-thyroidal hypermetabolism due to defective mitochondrial respiratory control.

In 1972, Olson et al(6) reported 7 cases of chronic progressive external ophthalmoplegia in whom skeletal muscle biopsy specimens showed red accumulations in the subsarcolemmal and intermyofibrillar regions with the modified Gomori trichrome stain. These irregularly shaped fibres were called "ragged-red". Oxidative stains and electron microscopy identified aggregates of abnormal mitochondria in these areas, mainly within Type 1 fibres. These histological findings gave new insight into the aetiology of the syndrome described by Kearns and Sayre (7) in 1958: an association of progressive ophthalmoplegia with retinal pigmentation, cardiac conduction defects and ataxia. Previous descriptions of this syndrome had been recorded, the first attributed to Von Graef in 1856. In 1968 Drachman (8) coined the term "ophthalmoplegia plus" to include all syndromes combining chronic progressive ophthalmoplegia with other forms of neuronal degeneration.

The 1970s brought confirmation of mitochondrial dysfunction with the identification of respiratory chain defects by a growing number of researchers (9,10,11). The clinical presentations of their patients covered a broad spectrum which included ophthalmoplegia, weakness, ataxia, deafness and growth failure, often accompanied by lactic acidosis. Maternal inheritance was suggested as a mechanism for Leber's Hereditary Optic Neuropathy (LHON) (12), a form of neuroretinal degeneration occurring in adolescent males.

Tsairis and coworkers (13) described a family with myoclonus epilepsy associated with ragged-red fibres in 1973. This was the first report of this combination of findings. In 1977, Shapira et al(14) proposed the term "mitochondrial encephalomyopathy" to describe complex multisystem diseases associated with structurally or functionally abnormal mitochondria in brain or muscle.

Publication in the same year of an article entitled "Lumping or Splitting? "Ophthalmoplegia Plus" or Kearns-Sayre Syndrome?" by Berenberg et al (15) provoked debate. The authors reviewed the clinical, biochemical and histological data of 5 new patients with ophthalmoplegia, retinal degeneration and heart block as well as 30 from the literature. They concluded that the findings in KSS were specific enough to warrant its classification as an independent entity, separate from the broad group of chronic ophthalmoplegias. Interestingly, persistent viral infection was considered a likely pathogenetic mechanism for KSS at that time.

This was followed in 1980 by Fukuhara's paper on myoclonus epilepsy associated with ragged-red fibres (MERRF)(16). In this he described 2 patients with myoclonus, convulsions, mental deterioration, cerebellar signs and muscular atrophy who had ragged-red fibres on muscle biopsy. The clinical findings were compared with those of other neurological disorders known to have mitochondrial structural abnormalities: Kearns-Sayre (Shy) Syndrome, Leigh encephalopathy, familial poliodystrophy (Alpers Syndrome) and Trichopoliodystrophy (Menkes disease). Genetic studies have since confirmed the existence of MERRF as a specific syndrome.
Later that year DiMauro (17) published a report on an infant who died at 3 months following progressive muscle weakness. This was associated with Fanconi syndrome and lactic acidosis. Although there were no ragged-red fibres, mitochondrial abnormalities were present on muscle biopsy and cytochrome c oxidase(cox) activity was less than 10% of normal in muscle and was reduced in kidney.

In 1984, Pavlakis and coworkers (18) reported 2 cases of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes, a syndrome for which they proposed the acronym "MELAS". The first patient had delayed motor and language development at 3 years, short stature at 8 years and sensorineural deafness at 14 years. At 15 he had 2 generalized seizures with residual weakness and sensory loss in the left arm. Computed axial tomography (CT) showed a right frontoparietal lucency. At 16 he was admitted to hospital with right hand weakness, confusion and aggression. Cerebrospinal fluid(CSF) protein was elevated at 0,76g/l. Bilateral non-enhancing parietal lucencies were present on CT scan

and cerebral angiography was normal. Resolution of the hemiparesis occurred within 2 weeks but 2 months later he presented with hallucinations and speech abnormalities. Again parietal lucencies were noted, arterial lactate was 2,7 millimoles/Litre (mM/L), CSF lactate 4,9 mM/L and ragged-red fibres(RRF) were seen on muscle biopsy.

The other patient had normal early development but there was a fall off in growth and school performance by 9 years when he presented with a right hemiparesis. This resolved over 4 weeks but 2 months later he developed sudden blindness accompanied by a generalised seizure. Bioccipital lucencies were seen on CT scan. The blindness improved but a left hemiparesis occurred 12 hours after a cerebral angiogram which had demonstrated right extracranial carotid occlusion. Lucencies were then present in the right basal ganglia and both occipital lobes. Investigation at 14 years showed an arterial lactate of 4mM/L, CSF lactate of 6,1 mM/L and ragged-red fibres.

Nine other children included in this study had similar symptoms with an onset between 3 and 11 years. On the basis of the similarities in clinical and laboratory findings in these patients, a new subgroup of mitochondrial diseases was proposed, the MELAS syndrome.

Thus by the mid 1980s 5 distinct clinical entities caused by mitochondrial dysfunction had been described; MELAS, MERRF, KSS, LHON and fatal infantile mitochondrial myopathy due to cytochrome c oxidase deficiency. This was in addition to the conditions mentioned by Fukuhara; Leigh, Alpers and Menkes syndromes. The number of reported cases grew steadily and it was recognised that there is a considerable degree of clinical overlap between syndromes. What followed was rapid progress in unravelling the genetics of these disorders facilitated by the advances in molecular biology which are revolutionising medical research in the 1990s.

The first documentation of a mtDNA point mutation was achieved by Wallace and coworkers (19) in 1988 in 9 of 11 families with LHON. This mutation resulted in the substitution of arginine for histidine in subunit 4 of complex 1 of the respiratory chain. Since then a number of other point mutations have been linked to LHON.

The next report of a mutation involving a structural gene was one affecting mitochondrial ATP synthetase (20). This occurred in association with a new clinical syndrome characterised by developmental delay, retinitis pigmentosa, ataxia and sensory neuropathy. This disorder has been given

the acronym NARP. When greater than 90% of the mtDNA is affected by the NARP mutation a fatal infantile neuropathy with the features of Leigh syndrome occurs (21).

An adenine to guanine point mutation at bp 8344 in the tRNA lysine gene was reported in 1988 by Wallace et al (22). This mutation has shown consistent correlation with the MERRF phenotype and in addition has been reported in association with Leigh Syndrome (23), myopathy or myoclonus with truncal lipomas and proximal myopathy (24). A second mutation at bp 8356 in the same tRNA lysine gene has been reported (25).

In 1990 an article in Nature by Goto and coworkers (26) described a mutation in the mitochondrial tRNA leucine gene, at bp 3243 and linked it to MELAS syndrome. A second mutation at bp 3271 on the same tRNA gene has since been reported (27). Both these point mutations have been shown to be maternally inherited. There is wider phenotypic variation with the tRNA leucine mutation than with the MERRF mutation, with some patients displaying myoclonus or ataxia in addition to features of MELAS.

In 1992, Reardon (28) reported a family with deafness, diabetes with low insulin levels and cardiomyopathy as the cardinal findings and a pattern of maternal inheritance. A bp A3243G mutation was identified by polymerase chain reaction (PCR) in the blood of all the diabetic patients tested as well as all offspring of female patients. No family members had neurological signs other than deafness. The relationship between this mutation and diabetes is not yet clear but diabetes and deafness are relatively common findings in relatives of patients with MELAS.

New mutations continue to be identified. Clarification of the relationship between mutation, biochemical defect and phenotype remains a challenge for the future.

CLASSIFICATION OF MITOCHONDRIAL DISORDERS

Classifications require regular updating. The biochemical classification of DiMauro (29) has been consistently useful.

BIOCHEMICAL CLASSIFICATION OF MITOCHONDRIAL MYOPATHIES

1.DEFECTS OF TRANSPORT

a)Carnitine palmitoyl transferase (CPT) deficiency
b)Carnitine deficiency
c)Defect of FAD uptake

2.DEFECTS OF SUBSTRATE UTILISATION

a)Pyruvate carboxylase deficiency
b)Pyruvate dehydrogenase complex deficiency
c)Defects of beta oxidation

3.DEFECTS OF THE KREBS CYCLE

a)Fumarase deficiency
b)Alpha ketoglutarate dehydrogenase deficiency

4.DEFECTS OF OXIDATION PHOSPHORYLATION COUPLING

a)Luft's syndrome

5.DEFECTS OF THE RESPIRATORY CHAIN

a)Complex I deficiency
b)Complex II deficiency
c)Complex III deficiency
d)Complex IV deficiency
e)Complex V deficiency
f)Combined defects of respiratory chain components

An additional genetic classification has been proposed by the same author.

GENETIC CLASSIFICATION

1.DEFECTS OF MITOCHONDRIAL DNA

a)Point mutations (maternal inheritance)
b)Deletions and duplications(sporadic)

2.DEFECTS OF NUCLEAR DNA (Mendelian Inheritance)

a)Defects in genes encoding mitochondrial proteins
b)Defects of mitochondrial protein importation
c)Defects of intergenomic communication

CLINICAL ASPECTS

This section will deal with aspects of clinical symptomatology relevant to the research study. Numerous clinical studies are now available for comparison but only a few of them concentrate on paediatric cases. Some of the more lethal conditions are seen virtually exclusively in neonatal or paediatric patients, but these are fortunately uncommon. The remainder may present at almost any age, the more severe defects tending to be diagnosed earlier. As pathology in mitochondrial disorders often evolves over many years, the diagnosis may not be evident in initial stages.

Apart from Luft's disease, which is very rare, the Kearns-Sayre syndrome and LHON were the earliest described clinical syndromes relating to mitochondrial dysfunction. LHON does not tend to present before adolescence and will therefore not be covered. MERRF, MELAS, Leigh, Alpers and PDH deficiency will be covered briefly.

KEARNS-SAYRE SYNDROME

KSS is a clinical syndrome with childhood onset, retinitis pigmentosa (RP) and chronic progressive ophthalmoplegia. In addition, one of the following is present: heart block, ataxia or an elevated CSF protein. Of Berenberg's 30 cases(15), all had onset before the age of 20 years, the youngest at three years. Ophthalmoplegia, preceded usually by ptosis, was the presenting symptom in 26 and pigmentary degeneration in 3. All had a degree of heart block although this was latent for prolonged periods in some patients. In addition to the cardinal features a number had short stature, impaired intellect, deafness, vestibular dysfunction, delayed puberty or weakness. Facial, palatal and neck weakness were also found as was a positive Babinski sign. CSF protein was elevated in 24/25 patients, the highest being 4g/l.

Ophthalmoplegia did not cause diplopia and was usually asymptomatic until eye movements were all markedly limited. Ptosis was asymmetrical in some patients. Pupillary responses were normal except for one case with a sluggish light reflex.

Retinal pigmentation was atypical with diffuse, fine stippling centrally and little effect on vision. 40% of patients had mild reduction in visual acuity or night blindness.

Retinopathy has been studied intensively by Mullie and coworkers (30). In 1985, they looked at 61 patients with mitochondrial myopathy, some of whom had KSS. 22 had a "salt and pepper" retinopathy with severe visual loss in 2. Two had atypical RP and 2 had loss of retinal pigmentary epithelium. These four were the most impaired. Electroretinogram (ERG) done in 11 patients with clinical retinopathy was abnormal in all, with reduced rod and cone response in 8.

Conduction blocks in Berenberg's patients ranged from prolonged interventricular conduction time to complete heart block and were the cause of death in 7 patients. The longest interval between the appearance of ophthalmoplegia and recognition of heart block was 36 years. Conduction disturbances increased in severity with time and once symptomatic required pacing. CSF protein may be used as a guide to which patients with progressive ophthalmoplegia require regular cardiac assessment.

Although no patient had seizures, nonfocal abnormalities on electroencephalogram (EEG) were found in 20/24 cases.

Seven of 9 patients had ragged-red fibres on muscle biopsy, 3 with normal electromyograms(EMG). Eight of 8 had abnormal mitochondria on electron microscopy(EM).

Postmortem examination done on 5 patients showed coarse vacuolisation within deep white matter, brainstem and spinal cord.

No family had more than one case of KSS indicating sporadic inheritance.

MYOCLONUS, EPILEPSY AND RAGGED-RED FIBRES (MERRF)

Clinical variation in this group is greater than in KSS. Previous reports consisted mainly of small group and family studies.

Fukuhara's original description was of 2 cases. The first, a 21 year old woman presented at 16 years with myoclonus and dysarthria. She was found to have coarse horizontal nystagmus, saccadic eye movements, scanning speech, intention tremor and ataxia. Tendon reflexes were absent, Babinski sign positive unilaterally and Romberg sign positive. She had pes cavus. She developed generalised seizures soon after presentation.

Muscle fibres showed variable size and ragged-red staining.

Her mother and brother both had abnormalities on EEG. The brother had a tremor of the hands and neck.

The second patient was a male who had developed dysarthria and myoclonus by 13 years. At 21 years he was found to have optic atrophy, marked horizontal nystagmus, scanning speech, ataxia and intention tremor. In addition myoclonus and generalized weakness were present with absent reflexes and positive Romberg sign. His muscle biopsy showed ragged-red fibres in 10% of fibres with moderate atrophy and interstitial fibrosis. He died a year later and autopsy revealed neuronal degeneration and gliosis in the subthalamic nucleus, striatum, globus pallidus, red nucleus and superior colliculus. These changes extended to the superior and inferior cerebellar peduncles, the dentate nucleus, the posterior columns of the spinal cord, the spinocerebellar tracts and anterior and posterior nerve roots.

On the basis of his findings, Fukuhara suggested that MERRF be recognised as a separate entity.

Rosing (31) described a large family in 1983 who had features similar to those of Fukuhara's patients. Some members were also deaf. They had spasticity with brisk reflexes in contrast to the others mentioned. Inheritance was clearly maternal but not X-linked.

Berkovic et al (32) examined 84 cases of progressive myoclonic epilepsy (PME) of whom 13 were diagnosed as having MERRF syndrome. Onset ranged from 3 to 62 years and within the same family from 4 to 59 years. Myoclonus was usually the initial symptom although psychomotor delay preceded this in 3 cases. Eight had tonic-clonic seizures which were easy to control and only one had intractable seizures. Muscle weakness tended to be mild. The progression of the disease was very variable. Cervical lipomas developing at around 40 years were noted in one family. Ragged-red fibres were seen in 6/8 biopsies. One had non-specific abnormalities and the other a normal muscle biopsy but abnormal clumps of mitochondria on skin biopsy.

Biochemical studies showed normal mitochondrial enzyme activity in 2 patients, decreased cytochrome c oxidase activity in 1, complex III deficiency in 1 and a generalised defect in the other two.

Positron emission tomography (PET) was carried out in these patients. Thirty eight cortical regions were examined. The mean cerebral metabolic rate for glucose in MERRF patients was 35% less than controls and that for oxygen 28% less. Cortical blood flow did not differ significantly. These findings were compatible with a defect in mitochondrial metabolism.

Eighty-five EEGs done on these patients showed slowing of background activity in 11/13, bursts of bilaterally synchronous delta activity in 10 and epileptiform abnormalities in 11. Bilateral massive myoclonus coincided with generalised spike and wave discharges in only one patient. The more common action myoclonus was not associated with EEG changes. Six of 13 patients had focal abnormalities and five had occipital spike or sharp waves. Slowing was mild to moderate and usually theta frequency. Three patients were photosensitive.

The 2 siblings who were the most severely affected of Berkovic's patients had pathological features of Leigh syndrome in addition to the system degeneration of MERRF. Their family has since been identified as carrying the 8344 tRNA lysine mutation. Apart from classical MERRF and Leigh syndromes, this mutation has been associated with adult onset myopathy, stroke-like episodes and progressive ophthalmoplegia. Individuals within one family may have different presentations owing to mitotic segregation and the threshold effect. Other factors as yet unidentified may also play a role.

MELAS

The patients reported by Pavlakis et al (18) have already been described in the historical review. As with MERRF, phenotypic variation due to the A3243G tRNA leucine mutation has become evident in recent years.

In 1983, Rowland (33) reported 11 MELAS patients, all of whom had normal early development and onset of symptoms between 4 and 11 years. Episodic vomiting associated with migrainous headache was relatively common. Recurrent cramping abdominal pain has also been described (34). Stroke-like episodes were sometimes delayed until the adult years with minor symptoms such as exercise intolerance being the only early indicators of disease(35).

Sensorineural deafness has been described in a number of patients. The identification of the A3243G mutation in families with maternally inherited diabetes and no central nervous system (CNS) manifestations has further demonstrated the complexity of the clinical syndrome. Some patients with MELAS have maternal relatives who have diabetes and/or deafness as the only clinical manifestation.

Fifty patients with MELAS were studied by Sakuta and coworkers (36). Clinical characteristics were tabulated with histological and biochemical findings. The A3243G mutation was found in 38. Of note is that episodic headache and vomiting were found in 86% of patients, convulsions in 81%, visual disturbances in 55% and transient hemiparesis in only 18%. These four symptom complexes comprised the "stroke-like" episodes. Weakness, mental handicap, short stature and deafness were the next most common with cardiomyopathy, heart block, ptosis and developmental delay occurring in a small number of patients.

37/38 (97%) had an elevated serum lactate and CSF lactate was elevated in all 35 tested.

Similarly, 37/38 had ragged-red fibres on muscle biopsy.

A Canadian study (37) of 10 paediatric patients documented onset between 2 months and 12 years in their group. None had a positive family history. Cortical blindness was common (4/10) and in contrast to the previous study 7/10 had hemiparesis, indicating a difference in patient selection. All patients developed cognitive deficit. All had increased serum lactate (4.5-9.8mM/L) and all had ragged- red fibres. As this was a selected group with well documented MELAS it is not surprising that the results were uniformly positive.

LEIGH SYNDROME

Leigh Syndrome is a well described neuropathological entity (38) with characteristic destructive lesions situated symmetrically in subcortical and brainstem structures. It was originally described in infants but is now known to affect older children and adults as well. Atypical cases with cortical involvement have been recognised. Usually three clinical stages occur: initial normal development; a second stage of motor regression including breathing abnormalities, pyramidal, extrapyramidal or cerebellar signs; a final stage which may be prolonged and manifest as extreme hypotonia, swallowing difficulties and convulsions. Blood and CSF lactate

levels are elevated. Previously the diagnosis was made at autopsy but advances in radiology now allow a reasonably confident diagnosis to be made prior to this in typical cases (39).

PYRUVATE DEHYDROGENASE DEFICIENCY

Pyruvate dehydrogenase (PDH) deficiency is an important cause of lactic acidosis in children. It is a complex enzyme with 3 catalytic components and a number of subunits. At least three genetic defects have been identified (40). Abnormality of the thiamine pyrophosphate pyruvate decarboxylase (E1) component is probably the most common (41). The gene for the faulty E1 alpha subunit is located on the X chromosome, Xp22.1. Clinical phenotype is characterised by severe, early onset lactic acidosis with seizures and apnoeic spells in male infants. Heterozygous females may present with neurological symptoms and mild lactic acidosis at a much later stage, depending on the pattern of X chromosome inactivation.

In 30 patients described by Robinson (42) hypotonia and psychomotor regression were common. Two patients had recurrent episodes of ataxia only. A number were dysmorphic with frontal bossing, wide nasal bridge, long philtrum and flared nostrils. Agenesis of the corpus callosum occurred in four of the severely affected group. The high number of female infants (4/10) with very low levels of enzyme activity is not explained. Genetic defects other than that involving the E1 alpha subunit are nuclear encoded and the male/female ratio of affected cases would therefore be expected to be equal.

ALPERS HUTTENLOCHER SYNDROME

A normal birth and neonatal period are followed by an insidious period of developmental delay, failure to thrive and onset of intractable seizures in this childhood neuronal degeneration. Liver disease is variable and may only present terminally (43). Onset is usually in infancy. EEG findings may be quite distinctive with marked slowing of high amplitude mixed with low amplitude polyspikes. Pathological findings in the liver consist of disorganization with regeneration, fibrosis, bile duct proliferation and microvesicular fatty change. Changes in the brain may be extensive with neuronal degeneration, astrocytosis, spongiosis and disorganisation. There is no biochemical marker for Alpers Syndrome but a mitochondrial aetiology has been proposed(44).

RADIOLOGY OF MITOCHONDRIAL DISORDERS

Improved scanning techniques, particularly the advent of magnetic resonance imaging (MRI) and functional imaging methods are continuing to add to our knowledge of mitochondrial pathology.

Characteristic of the lesions seen in MELAS is that they cross vascular boundaries and are more common in the parieto-occipital regions. The process responsible for these radiological appearances remains uncertain. They appear as transient, low density regions on CT scan (35), at times appearing after an episode of status epilepticus. They may be multifocal at onset or involve different regions at different times and they usually involve cortical grey matter. Resolution often occurs but is followed by further changes. Generalised atrophy and basal ganglia calcification are not uncommon. Rare cases of massive focal brain swelling with midline shift have been reported (45).

Mathews (46) in 1991, published a report of three MELAS patients who had fairly specific MRI abnormalities. Their patients showed laminar hyperintense signals on T2 weighted scanning, consistent with oedema or focal cortical necrosis. Cortical grey matter and adjacent white matter were affected.

Recent studies of cerebral blood flow in MELAS by Ooiwa (47) were published in 1993. Lesions were identified on CT and MRI and were not enhanced with gadolinium. Cerebral angiography, when done at the time of the stroke-like episodes, showed dilated cortical arteries and focal areas of early venous filling. Cerebral blood flow was evaluated by dynamic and xenon enhanced CT within 72 hours of onset of the stroke and found to be increased in affected areas. Serial studies failed to show an ischaemic area at any time throughout the patients' illness. Interpretation of these findings suggests that metabolic dysfunction is responsible for the lesions rather than ischaemia.

Evidence of metabolic abnormality has also been demonstrated using magnetic resonance spectroscopy (MRS) in four patients with KSS (48). Compared with controls they had higher basal lactate levels in the visual cortex and did not show transient elevation in response to photic stimulation.

Radiological changes in MERRF are less prominent. Many patients have normal scans or exhibit a degree of cerebral atrophy. Low density areas within cerebral or cerebellar white matter may be seen. KSS may have

similar changes with involvement also of subcortical and brainstem structures. Increased signals due to iron or calcium deposition in the basal ganglia are also found.

Positron emission tomography (PET) in MERRF patients measuring the cerebral metabolic rate for oxygen and glucose in addition to cerebral blood flow (CBF), showed a 30% reduction in these rates when compared with controls (49). This was in the presence of normal cerebral blood flow. These findings are consistent with a chronically low rate of oxidative metabolism.

Phosphorus magnetic resonance spectra (MRS) can be used to determine the concentrations of adenosine triphosphate (ATP), phosphocreatine (PCr), and inorganic phosphate in muscle. From these, adenosine diphosphate (ADP) levels and the phosphorylation potential of cells can be calculated, giving a sensitive indicator of mitochondrial function (50).
These various functional techniques are becoming increasingly useful in mitochondrial research as they allow study of metabolic changes in vivo.

HISTOPATHOLOGY

Muscle tissue more than any other has been studied in these patients as it is accessible and susceptible to changes in oxidative metabolism. The pathological hallmark of mitochondrial disorders is the presence of ragged-red fibres when using the Gomori trichrome stain [fig.1]. These are fibres with intense red subsarcolemmal staining and a "moth-eaten" appearance. They are usually present in Type 1 fibres. They are not entirely specific for mitochondrial disease but are very suggestive if present in large numbers. Additional non-specific findings on light microscopy include atrophy, interstitial fibrosis and fibre hypertrophy (16). Ragged-red fibres contain more abundant mutated mtDNA and usually lack COX activity (29). Overall protein synthesis appears reduced in RRF possibly due to a depletion of functional tRNAs. They are commonly found in patients with deletions and point mutations affecting tRNA but not in those with mutations of structural genes (LHON).

The histochemical stains, succinate dehydrogenase (SDH) and COX allow better interpretation of light microscopy findings. SDH, a Kreb's cycle enzyme is a sensitive marker of ragged-red fibres and COX deficiency is an indicator of biochemical dysfunction.

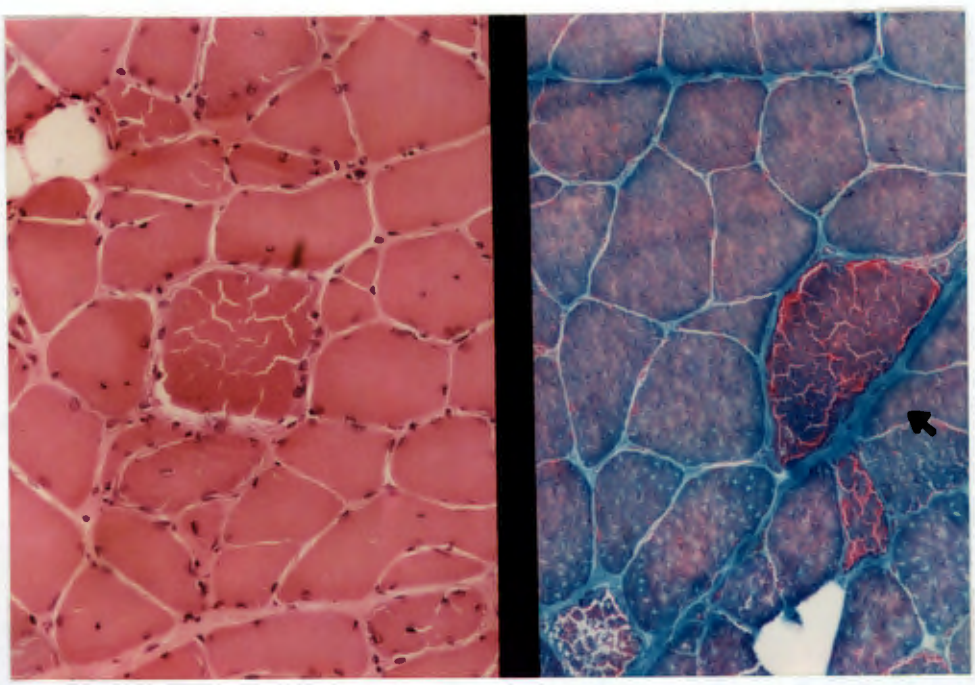

Figure 1. Gomori Trichrome stain of a muscle biopsy taken from case 10, showing ragged red fibres.

More recently, application of immunological techniques has allowed comparison of nuclear and mitochondrially encoded protein synthesis in individual fibres by using antibodies directed against different subunits of the respiratory chain (51,52).

Electron microscopic findings are variable. They include enlargement of mitochondria, bizarre morphology, paracrystalline inclusions, internal tubular structures and various abnormalities of cristae formation. These findings again are suggestive in the appropriate clinical context but are not specific. Similar changes have been induced experimentally by ischaemia and toxin exposure.

A comprehensive review of the neuropathology of mitochondrial disorders has recently been published (53). KSS is a multisystem disorder characterized by spongiform encephalopathy, neuronal degeneration, astrocytosis and demyelination. MERRF tends to select myelinated tracts and MELAS has typical features of multifocal necrosis, mineral deposits, neuronal degeneration and spongiosis.

BIOCHEMICAL STUDIES

These concentrate on defects of the respiratory chain although the broad classification of mitochondrial disorders includes pyruvate dehydrogenase deficiency and carnitine disturbances amongst others. These assays should be done in appropriate circumstances.

The respiratory chain consists of four multiprotein complexes spanning the inner mitochondrial membrane. Electrons are transported along this chain through a series of reduction reactions producing oxygen and a large amount of energy which is coupled to the formation of ATP. Pyruvate and free fatty acids serve as substrates for this process.

The first of these protein complexes, Complex I (NADH coenzyme Q reductase) contains 26 polypeptides, 7 of which are coded for by mtDNA. Complex II (succinate coenzyme Q reductase) is coded for by nuclear DNA; Complex III (reduced coenzyme Q cytochrome c reductase) has 1

mitochondrially coded subunit and Complex IV (cytochrome c oxidase) has three. Adenosine triphosphate synthetase (ATPase) or Complex V has two subunits coded by mtDNA.

Many patients have now been reported with defects of respiratory chain function arising from genetic mutations in mtDNA.

Referring to DiMauro's classification (29), MERRF, MELAS, KSS and LHON mutations may all cause defects in Complexes I, III and IV. Individuals may have partial deficiency of one or all of these enzyme systems. The same complexes are affected in maternally inherited deafness, diabetes and cardiomyopathy and in congenital fatal myopathy.

Leigh syndrome has been associated with cytochrome c oxidase (cox)(39) and ATPase deficiency, in addition to PDH deficiency (40). COX deficiency has also been identified in Alpers syndrome, fatal and benign infantile myopathy, CPEO and Menkes disease.

Correlation between the severity of disease and the degree of biochemical deficiency has been shown in several studies but this is not consistent. Heteroplasmy may cause equivocal results. Phenotypic variation seems to depend in part on the tissue affected and the degree of enzyme deficiency but clarification on these issues is still required.

MITOCHONDRIAL GENETICS

Refer [fig 2].

Mitochondrial deletions:

Large single deletions are found in KSS, sporadic progressive external ophthalmoplegia (PEO) and Pearson Marrow/Pancreas Syndrome. The latter consists of a refractory sideroblastic anaemia and exocrine pancreatic dysfunction.

Deletions tend to involve the larger arc of mtDNA between the two origins of replication (54). It is thought that these deletions arise during oogenesis. Approximately 50% are flanked by direct repeats 5 to 13 base pairs in length. The deletions are primarily found in muscle with only very small amounts present in fibroblasts and leucocytes. This suggests selection against genetically defective mitochondria in rapidly dividing cells.

Deletions usually affect a number of tRNAs thereby causing diffuse biochemical defects. No clear correlation between the site of the biochemical defect and the clinical signs has been established . Patients with apparently identical deletions have been found to have different biochemical abnormalities and clinical features (54). Where localisation of deficiency occurs and the biochemical defect is less than expected it is thought that sharing of tRNAs between normal and abnormal genomes within the same mitochondrion may occur (55). The proportion of normal to mutant mtDNA is therefore important in determining clinical outcome.

Multiple deletions have been described with an autosomal dominant inheritance pattern and in sporadic cases(56).In these cases it seems likely that a faulty nuclear gene affects mitochondrial repair mechanisms.

MERRF and the tRNA lysine mutation:

The A to G point mutation at bp 8344 in the tRNA lysine gene was described by Wallace et al in 1988 (22). This causes a diffuse biochemical defect and is heteroplasmic as homoplasmy would be incompatible with survival. Normal mtDNA has a protective effect and a decline in protein synthesis occurs when levels fall below 15%. Large changes in phenotype can then occur with only small changes in genotype. In the many families which have now been reported, correlation between genotype and phenotype is not always consistent. The family reported by Graf (57) showed 85% mutant muscle DNA in a mother with marked weakness and 92% with normal morphology in her child with minimal symptoms. Age may have some bearing on this but does not provide a satisfactory enough explanation.

The A8344G mutation accounts for 80 to 90% of MERRF cases. A new mutation at bp 8356 of the tRNA lysine has recently been described (25).

MELAS and the tRNA leucine mutation:

Most patients with typical MELAS have an A3243G mutation in the tRNA leucine gene. Some have one at bp3771 in the same gene. The proportion of mutant mtDNA is much higher in symptomatic cases than in their oligosymptomatic relatives, though it is commonly found even in asymptomatic family members. Again the percentage in muscle is much higher than in blood and the mutation causes a generalised biochemical defect. Diabetes and deafness are often found in family members with the mutation and a family with maternally inherited diabetes without encephalopathy has been described (28).

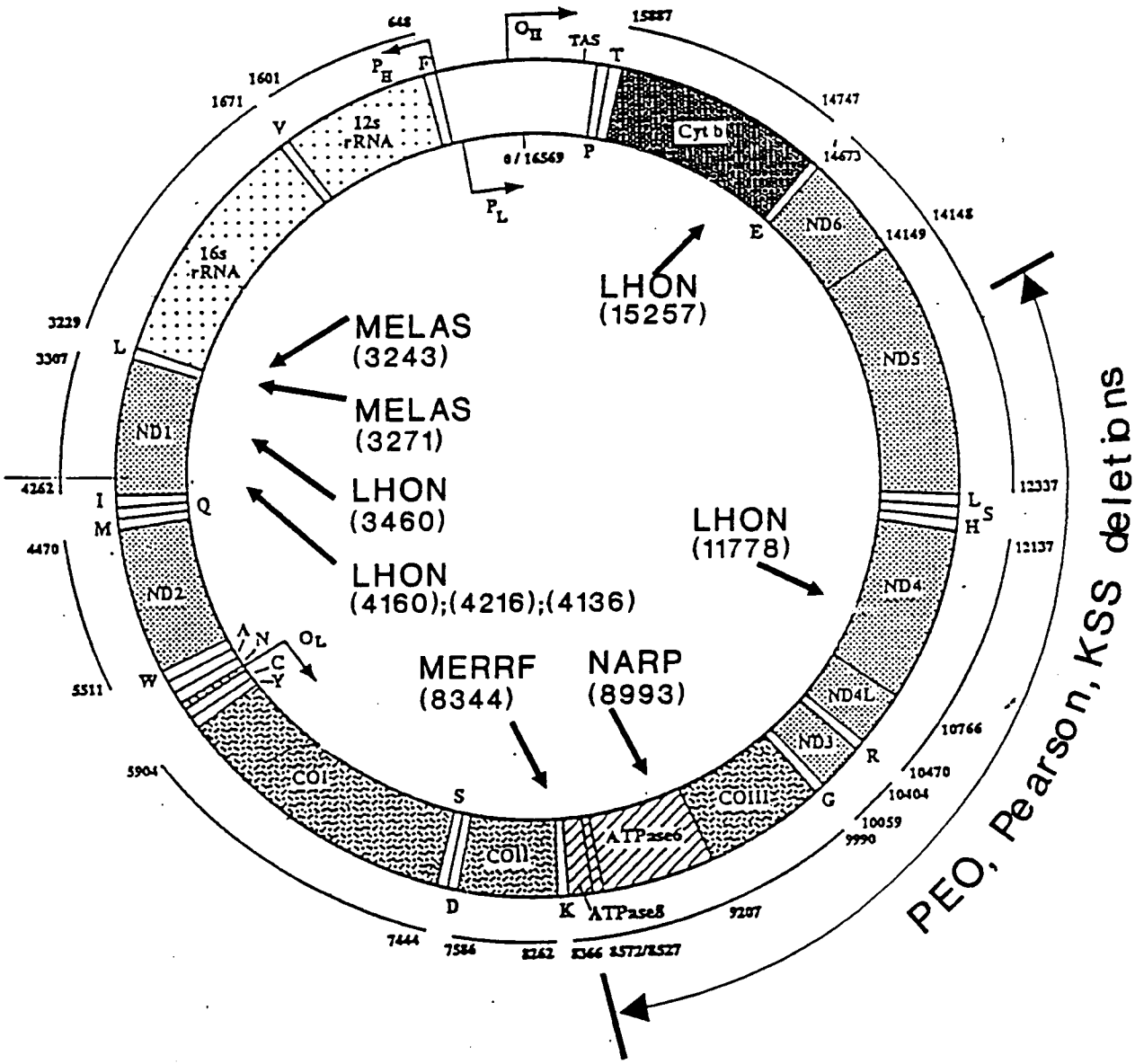

Figure 2. Diagramatic representation of mitochondrial DNA demonstrating some of the known sites of mutation.

A subgroup of patients with the bp3243 mutation have PEO without stroke and this should be considered in those patients with PEO but without deletions.

In conclusion a great deal has been learned about mitochondrial disorders in recent years. Diagnosis is becoming easier and it is hoped that further research will benefit patients in terms of counselling and therapy.

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MITOCHONDRIAL ENCEPHALOMYOPATHIES

AT THE RED CROSS CHILDREN'S WAR MEMORIAL

HOSPITAL

AIMS

A number of children followed up at the Red Cross Children's Hospital Neurology Clinic have had unusual presentations associated with hyperlactataemia. Many of these had histological changes in skeletal muscle suggestive of mitochondrial dysfunction.

It was proposed to study the clinical, biochemical and histological data of these children. Such a data base would give insight into the spectrum of clinical pathology in the local population. It would provide documentation of unusual cases and critical evaluation of the criteria upon which the diagnosis of mitochondrial encephalomyopathy was based. Coordination of family and patient information would indicate appropriate biochemical and genetic investigations.

METHODS

INCLUSION CRITERIA

Criteria for inclusion in the study were:

a)An unexplained neurological or neuromuscular disease with either (b) or (c)

b)Unexplained elevation in serum lactate > 1,8 mM/L.

c)Abnormal mitochondrial morphology on either light or electron microscopy.

METHODS

Children with mitochondrial encephalomyopathies are not readily identifiable through the hospital record system as there is no diagnostic code for the condition. Cases are therefore coded according to symptoms.

The names of children included in the study were supplied by clinicians working in the Neurology Service. The children selected were interviewed with their parents to obtain a detailed history of the illness and of any relevant family history. General, neurological and ophthalmological examinations were performed, the latter by arrangement with the Department of Ophthalmology.

Laboratory investigations included the following:

1.BIOCHEMICAL:

Electrolytes, renal and liver function tests
Calcium, magnesium, inorganic phosphate
Creatine kinase
Random blood glucose
Lactate, pyruvate
Cerebrospinal fluid (CSF) lactate*
Ammonia*
Alanine*
Amino & organic acids*
Copper, caeruloplasmin*
Pyruvate dehydrogenase*, pyruvate carboxylase*

*done on selected patients

A few patients had further specialised investigations done depending on symptomatology. These included arylsulfatase, very long chain fatty acids (VLCFA), carnitine levels (1 patient, assay not routinely available), thyroid function and mucopolysaccharide screens.

2.ELECTROPHYSIOLOGY:

Electroencephalogram (EEG)
Electroretinogram (ERG)
Visual evoked responses (VER)
Brainstem auditory evoked responses (BAER)
Nerve conduction studies (NCS)
Electromyogram (EMG)

3.RADIOLOGY:

Computed axial tomography (CT)
Magnetic resonance imaging (MRI)

4.ELECTROCARDIOGRAM (ECG):

5.MUSCLE BIOPSY:

This was done under general anaesthesia. Specimens were taken from the deltoid or quadriceps femoris muscle and divided for light and electron microscopy. Routine staining included haematoxylin and eosin, the modified Gomori trichrome, ATPase, NADH and special stains for lipid in some cases.

RESULTS

37 children fulfilled the criteria for entrance into the study. Of these 30 were physically examined by the author, six were unavailable for study and 1 had died. Data for these latter seven were obtained from hospital files. Four other children died during the study period. Causes of death will be further discussed.

The follow up period at Neurology Clinic varied from 2 months to 10 years, with a mean duration of 3 years 10 months.

Subjects were between 9 days and 18 years of age with a median age of 7 years 9 months. Age of onset of symptoms ranged from birth to 8 years. Twenty had symptoms before 2 years, eleven between 2 and 6 years and six after the age of 6 years.

All the children who died presented in the first year of life. The oldest age at presentation was 8 years. Any child presenting beyond the age of 12 years would not have been referred to our clinic, but rather to other teaching hospitals.

PERINATAL ABNORMALITIES

Perinatal complications were common but there was only one documented case of asphyxia neonatorum. Three patients had body mass of < 2500 grams(g) at birth and one was < 1500g. Ten had been born by Caesarean Section (CS); 4 of these were elective, 2 for abnormal fetal presentation, 2 for cephalopelvic disproportion (CPD) and 2 for fetal distress. There were 2 instrument deliveries and single cases of meconium stained liquor,

hypoglycaemia, exchange transfusion and prolonged neonatal jaundice. Four infants were hypotonic post delivery and one required ventilation (case 12,Table 4).

Detailed family histories were difficult to obtain in some cases. There were 2 sibling pairs, one with mental handicap and seizures, the other with mental handicap and abnormal eye movements. The latter pair had different fathers, supporting maternal inheritance. A number of children had relatives with mental handicap, seizures, diabetes mellitus, deafness and muscle cramps but maternal inheritance was not clearly demonstrated.

CLINICAL FINDINGS

Findings are summarised in Tables [1-4]. Individual cases of interest will be discussed in the text. Case numbers refer to Table 4.

Twenty eight children fulfilled all 3 inclusion criteria. Two had normal lactate values with abnormal histopathology and seven did not have a muscle biopsy performed.

Twelve patients had height on the 3rd centile or below. No child was under 60% of expected height. None of the children had clinical evidence of retinopathy, deafness or heart block. Renal and endocrine abnormalities were also absent. Peripheral neuropathy occurred in two patients. 6 children had mild dysmorphic features, but none had identifiable syndromes or chromosomal abnormalities.

There were two principal patterns of clinical presentation: encephalopathy and myopathy. The diagnosis of encephalopathy was based on loss of motor function and or intellectual capacity combined with the appearance of abnormal neurological signs. Patients in the myopathic group showed weakness with hypotonia or ophthalmoplegia. Thirty patients had predominant encephalopathy and seven showed a myopathic picture. Seven of those with encephalopathy had global delay and three with myopathy had delayed motor milestones.

ENCEPHALOPATHIC GROUP

Presenting symptoms in the encephalopathy group were very variable. Seizures and developmental delay or regression were the most common.

ENCEPHALOPATHY GROUP

PRESENTING SYMPTOM	NUMBER OF PATIENTS
SEIZURES	15
STROKE-LIKE EPISODES	4
MOVEMENT DISORDERS	4
DEVELOPMENTAL DELAY	3
WEAKNESS	2
FAILURE TO THRIVE	1
ABNORMAL EYE MOVEMENTS	1
	TOTAL=30

Table 1.

MYOPATHY GROUP

PRESENTING SYMPTOM	NUMBER OF PATIENTS
WEAKNESS	4
HYPOTONIA	2
OPHTHALMOPLEGIA	1

Table 2.

CLINICAL FEATURES OF STUDY GROUP

CLINICAL FEATURES	NUMBER OF PATIENTS
DEVELOPMENTAL DELAY	26
SEIZURES	18
SHORT STATURE	12
MYOPATHY	9
ATAXIA	8
MYOCLONUS	5
STROKE-LIKE EPISODES	6
MOVEMENT DISORDERS	5
OPHTHALMOPLEGIA	3
OPTIC ATROPHY	2
DYSMORPHISM	3
PERIPHERAL NEUROPATHY	2

Table 3.

Table 4. Case summaries according to the numbers used in the text. Abbreviations used in the clinical signs column: M myoclonus, E epilepsy, D developmental delay, SS short stature, A ataxia, P pyramidal signs, W weakness, O ophthalmoplegia, EP extrapyramidal, S stroke, PN peripheral neuropathy. Abbreviations used in the microscopy column are ND for not done, S for subsarcolemmal accumulations of mitochondria, R ragged red fibres, N/S nonspecific. + abnormal result, - normal result.

CASE NUMBER	AGE OF ONSET	CLINICAL SIGNS	LACTATE LEVELS	RATIO LACTATE / PYRUVATE	INCREASED CSF LACTATE	LIGHT MICROSCOPY	ELECTRON MICROSCOPY	ELECTRO-RETINOGRAM	EEG	CT SCAN	MRI	PDH
1	3.50	M,E,D,SS,A	2.9	25	ND	-	+	-	+	-	+	-
2	.50	E,D	1.2	ND	-	S	+	+	+	-	+	ND
3	2.25	M,E,D,P,SS,A,W	4.7	22	ND	-	-	+	+	-	ND	-
4	.50	D,P,SS,W	4.6	35	+	-	+	+	+	+	ND	+
5	.50	E,D,P,W	6.8	35	+	-	+		-	-	ND	ND
6	UNKNOWN	E,W	2.3	20	ND	-	+			-	ND	ND
7	8	O,SS,EP	2.9	37	ND	ND	ND	+	+	+	ND	-
8	.50	E,D	3.4	39	ND	S	+	-	+	-	ND	ND
9	1	E,D,W	1.5	ND	ND	-	+	-	+	-	ND	ND
10	BIRTH	D,W	3.9	8	ND	R	+	+	-	ND	ND	-
11	.90	E,D,S,A,EP	4.6	25	-	S	+	ND	-	-	+	-
12	BIRTH	W	34.0	60	ND	LIPID	+	ND	ND	ND	ND	ND
13	O.5	EP	4.9	37	ND	S	+	ND	-	-	ND	-
14	1.50	E,D,SS,W	2.7	36	ND	S	+	B/L	+	-	ND	ND
15	6	M,E,D,O,S,SS	4.1	53	ND	S	+	+	+	-	+	ND
16	BIRTH	E	3.0	25	ND	ND	ND	ND	+	ND	ND	ND
17	1	D,O	4.0	22	ND	R	+	-	-	-	ND	ND
18	2.50	A,EP	2.4	23	-	-	N/S	ND	ND	+	-	ND
19	3.50	M,E,D,S	7.0	40	ND	-	+	-	+	-	ND	-
20	BIRTH	E,D,A,EP	2.7	22	ND	S	+	+	+	-	ND	-
21	BIRTH	D	3.0	45	ND	ND	ND	ND	ND	+	+	ND
22	2.60	S,P,SS,W	2.7	18	+	ND	ND	-	ND	+	+	-
23	2	S,P,A	2.2	22	-	S	+	-	+	-	-	-
24	5	SS,A,W	2.8	33	+	S	+	ND	ND	-	-	ND
25	3	E,SS	10.0	48	ND	ND	ND	-	+	-	ND	-
26	BIRTH	E,D,EP,W	2.5	31	+	S	N/S	-	+	-	+	ND
27	BIRTH	E,D,P,W	3.1	18	+	-	+	+	+	+	ND	-
28	BIRTH	M,E,D	2.7	29	ND	ND	ND	+	+	+	ND	-
29	2.75	D,S	2.7	46	+	-	+	-	+	-	+	-
30	7	E,D,P,SS	2.7	20	-	-	+	-	+	-	-	ND
31	7.25	D,S,P,SS	2.7	25	+	ND	ND	-	+	-	+	-
32	4	S,EP	2.9	26	+	S	N/S	ND	+	+	+	±
33	6.75	O,W	2.4	23	ND	S	+	ND	+	-	+	ND
34	BIRTH	D,A	3.3	22	ND	-	N/S	+	-	-	ND	-
35	.25	D,W	2.0	37	ND	S	+	-	+	ND	ND	-
36	3.80	D,O,W,PN	10.6	19	ND	S	+	+	-	+	ND	+
37	.66	M,E,D,PN	3.0	23	ND	S	-	-	+	+	+	-

Six children had stroke-like episodes. Their clinical histories will be presented in some detail.

Case[31]: MELAS syndrome.

This boy had his first episode at 7 years. He had been born prematurely, weighing 1300g and was diplegic with mild mental handicap. His mother suffers from a psychiatric disorder and after her first pregnancy developed sensorineural deafness and
non-insulin dependant diabetes mellitus. Her older male child is short and has some learning difficulties.

At the onset of his illness he was found unconscious in bed. On examination it was noted that he was of short stature and drowsy with generally increased tone and reflexes, especially in the lower limbs. As he recovered it became apparent that he was cortically blind. CT scan showed a low density lesion in the left occipital lobe. Vision recovered slowly over several months. He remained well until 9 years when he suddenly became acutely confused. EEG showed generalised slowing with temporal sharp waves. CSF protein was 1g/l and lactate 4.4 mM/L with a serum lactate of 2.7mM/L. He recovered within 3 days with mild residual disorientation, but 2 weeks later again developed acute confusion, headache and photophobia. He had a right sided constructional apraxia with receptive and expressive dysphasia and verbal perseveration. At this stage a CT scan was done: it showed generalised cortical atrophy. Cerebral angiography was normal. On MRI scan a large, left sided low density lesion was seen involving the parietal and occipital lobes [fig.3]. Testing DNA from circulating white cells showed the bp 3243 tRNA leucine mutation thus confirming the diagnosis of MELAS syndrome.

Recovery from the apraxia and speech disturbances was virtually complete a month after the second episode but intellectual function has deteriorated since.

Family members have not agreed to be tested but it is almost certain that his mother carries the mutation and has a milder form of expression.

Five other children had stroke-like episodes but did not have the typical features of MELAS. Only two thus far have had repeated episodes. Their details are as follows:

Case [23]

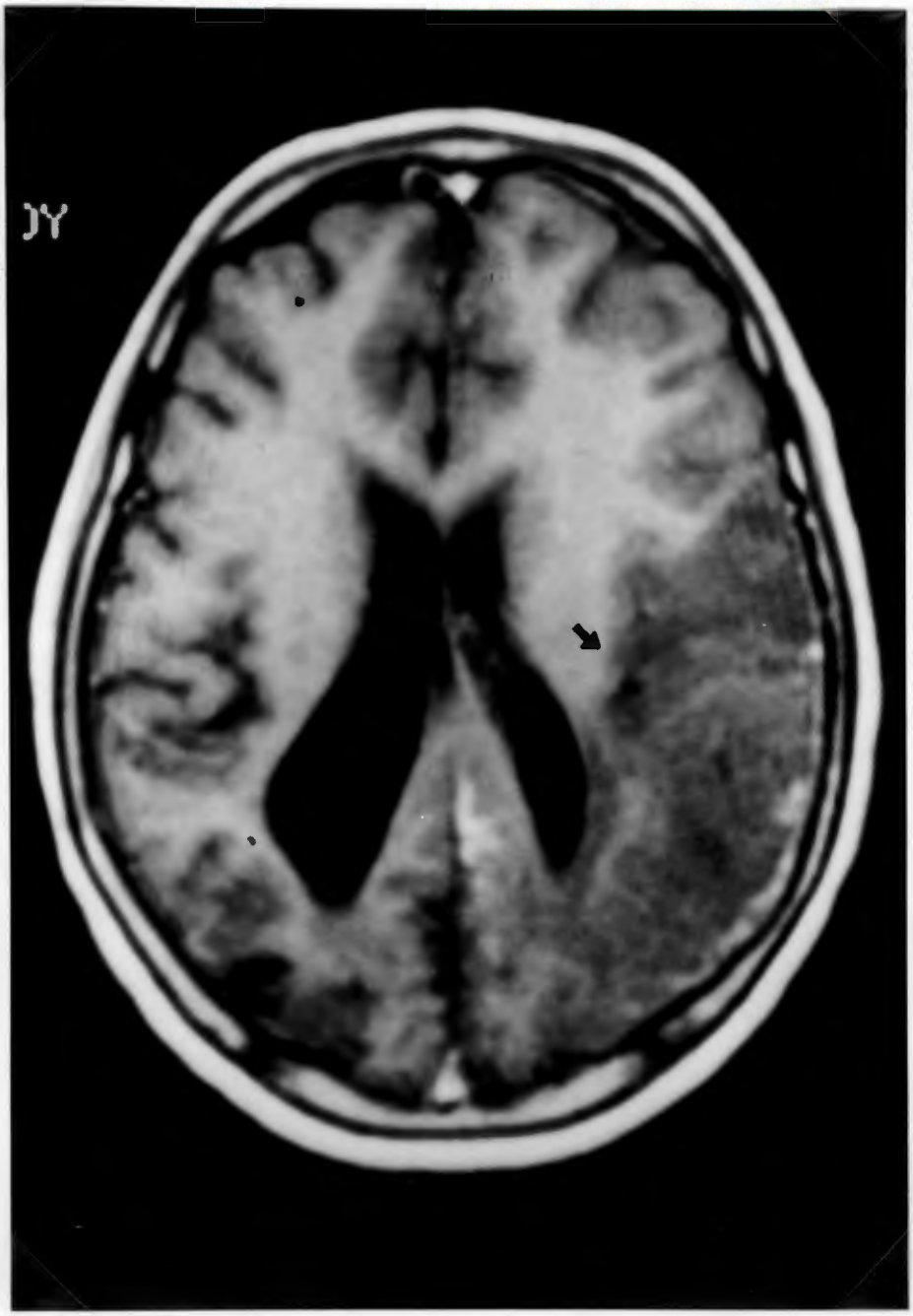

Figure 3. MRI scan of patient 31 (MELAS syndrome) demonstrating low intensity signals in the left parietal and both occipital lobes. Atrophy of the right occipital lobe is present with ex vacuo dilatation of the posterior horn of the right lateral ventricle.

This child had intermittent episodes of limb weakness starting at the age of 2 years. Later speech and swallowing became impaired. He progressed to spastic quadriparesis with pseudobulbar palsy and is in a wheelchair at 18 years. Compared with his motor impairment, intellectual deterioration has been slight and growth normal. There is no family history. CT and MRI scans have been negative repeatedly. CSF lactate was at the upper limit of normal at 1.8 mM/L and serum levels marginally elevated, 2.2 mM/L. EM of skeletal muscle showed large mitochondria with whorled cristae.

Case [29]

At 2.5 years of age, this patient developed rapid onset of generalised weakness and reduced level of consciousness over 5 days. She was hypotonic but had brisk reflexes. CSF was normal apart from a lactate of 4.6 mM/l. Serum lactate was 2.7 and L/P ratio was 46. CT head was normal but on MRI there were low density areas in the basal ganglia, external capsule and brainstem. EEG showed marked generalised slowing. On muscle biopsy there was evidence of lipid storage but no specific mitochondrial changes. She recovered over several weeks but remains mentally handicapped.

Case [22]

Case [22] developed normally until 2.5 years when he experienced sudden onset of right sided weakness followed by rapid progression to complete quadriparesis with pseudobulbar palsy. CT and MRI demonstrated large confluent plaques of demyelination involving predominantly the centrum semiovale, similar to the changes seen in Schilders disease(fig.4). Basal ganglia lesions were noted on a follow up scan 3 months after the onset of the illness. VLCFA and arylsulfatase levels were normal. CSF had elevated lactate at 3.04mM/L. He made good recovery but has residual spasticity and mild mental handicap. No subsequent episodes have occurred over three years and follow up scans have shown slight resolution of the original lesions.

Case [32]

This patient developed focal dystonia involving his left leg and later the left arm. CT showed a low intensity lesion in the right putamen. MRI demonstrated a second area in the left cerebellar peduncle. Serum lactate was 2.9 mM/L,/P ratio 26, CSF lactate done at a later stage was 2.5 mM/L. There was some peripheral accumulation of stain on the Gomori but no RRF. Although seizures were not a feature, the EEG showed

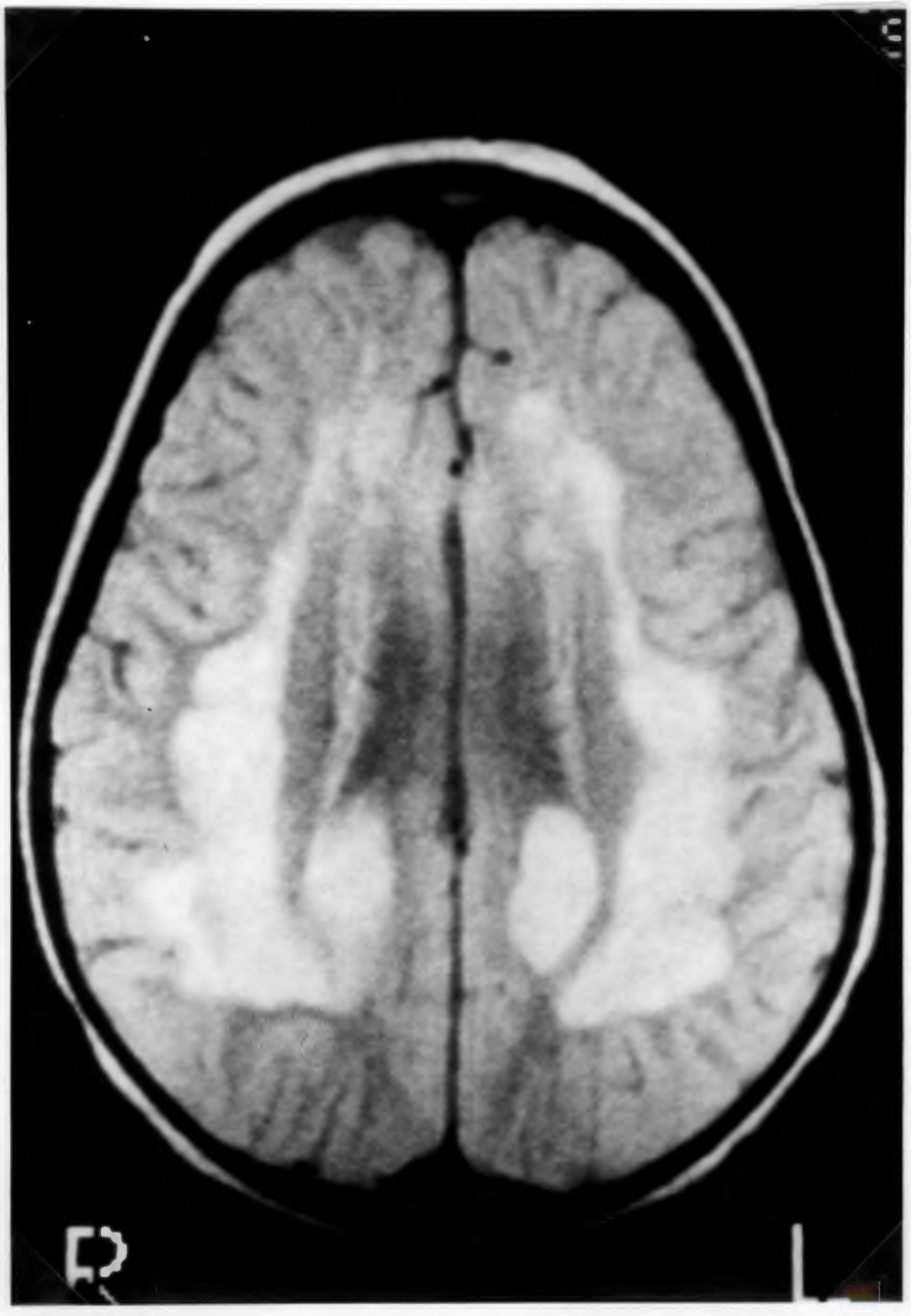

Figure 4. MRI Scan of the cranium of case 22 demonstrates large confluent plaques of demyelination.

generalised slowing with a spike and wave paroxysm. Dystonia responded to a combination of benzhexol and carbidopa. There was no evidence of a vascular or infectious aetiology and Wilson's disease was excluded.

Two cases had an overlap syndrome with features of both MERRF and MELAS.

Case [15]

This child deteriorated scholastically at 8 years. His parents are first cousins and he has a younger sibling with unilateral ptosis. He developed atypical absences with a diffuse epileptiform pattern on EEG. Myoclonic jerks, cerebellar signs and visual disturbance with optic atrophy were also found. A year after presentation he was admitted with a metabolic acidosis, pH 7.07 BE -17 SB 11 and serum lactate 5.9 mM/L with a lactate/pyruvate ratio of 53. MRI showed T2 hyperintensity in parietal paraventricular white matter as well as in the left occipital horn. CSF lactate measured 2 weeks after the acute admission was 3.1 mM/L. Since then there has been slow deterioration mentally and poor growth. Generalised, myoclonic and partial seizures have all occurred and have been resistant to therapy. On 2 occasions he has had seizures followed by hemiparesis which has lasted several days. Muscle biopsy showed increased subsarcolemmal staining on the Gomori and enlarged mitochondria on EM, but the findings were non specific. He has had periods of remission lasting up to a year and is ambulant 6 years after the onset of his illness.

Case [19]

Case 19 also showed a mixed picture. He had apparently uncomplicated Haemophilus Influenzae meningitis at 6 months. At 1 year he developed akinetic drop attacks. These were initially infrequent but increased and were resistant to therapy with valproate and benzodiazepines. From the age of 4 years there was progressive deterioration in his intellectual status and he began to walk with a broad base. CT scan was normal. At 8 years he was found unconscious in bed following a mild respiratory infection. There were no focal neurological signs. He had right sided pneumonia and required ventilation for poor respiratory effort. Serum lactate was 6.8 mM/L with a lactate/pyruvate ratio of 40. Transaminases and ammonia were marginally raised and valproate was discontinued. EEG showed marked cortical suppression. On muscle biopsy there was excessive lipid accumulation. Repeat CT showed no changes. He remained unrousable for a week before making a slow recovery.

MERRF-like syndrome

Apart from cases 15 and 19, three other children(cases 1,3,28) had myoclonus, generalised seizures and intellectual deterioration associated with elevations in serum lactate levels. Cases 1 and 3 had progressive myoclonic epilepsy with short stature, mild dysmorphism and intermittent ataxia. Case 28 had progressive myoclonic epilepsy (PME) with poorly defined, low amplitude responses on ERG. Other causes of PME were excluded.

Myoclonus was mild and could be controlled with valproate or benzodiazepines. None of the three had deafness or a positive family history. Cases 1 and 3 had poorly defined VERs and ERGs showed increased latency and low amplitude responses in cases 3 and 28. Generalised delta activity interspersed with sharp wave activity was also found on EEG in cases 1 and 3. Case 28 had intractable seizures and records performed during episodes of minor motor status epilepticus showed continuous high amplitude spike waves.

Lactate levels were elevated in all three, independent of ictal activity. Lactate/pyruvate (L/P) ratios were 25, 22 and 29 respectively.

Muscle biopsies showed large, abnormal mitochondria with whorled cristae and angular inclusions in case 1. Case 3 showed only minimal changes and case 28 did not have a muscle biopsy performed.

PDH DEFICIENCY

Two patients were diagnosed as PDH deficient. Case 4 had clinical features consistent with a severe defect, with onset of failure to thrive and developmental fall off from 6 months of age. She had mild dysmorphic features similar to those described in the literature(58). Her course was relentlessly progressive with recurrent vomiting, ataxia and increasing weakness. She died following an admission to hospital in shock with lactate levels of 8.3 and 12 mM/L.

The other patient, case 36, had a more atypical course. He presented with painless, symmetrical distal weakness at three years of age and was found to have a sensorimotor neuropathy, confirmed by nerve conduction studies. In addition he had limited upward and lateral gaze. Lactate was 10.6mM/L, pyruvate 555uM/L and the L/P ratio 19. Acidosis was not present although there was some respiratory compensation (pH 7.39; CO2 32; BE -3.9; SB 21). There was gradual progression with increased limb girdle weakness

but ambulation was maintained. Ataxia was present, disproportionate to the weakness. EMG was indicative of a neuropathy and ERGs were markedly reduced bilaterally in the absence of overt retinopathy. This patient remained stable enough to attend a school for the physically disabled. Growth was normal.

He presented at 9 years with sudden increase in weakness and ataxia, so that he could not walk unaided. He was confused, had staccato speech, bilateral ptosis and virtual complete ophthalmoplegia. No signs of infection were present. He was commenced on thiamine 600mg daily and was back to his previous level of function within 4 days.

LEIGH SYNDROME

Case 26 had a clinical course and radiological features compatible with Leigh syndrome. She developed normally for the first months of life but at 6 months became hypotonic, lost head and trunk control and developed strabismus. She had intermittent tonic episodes thought to be seizures. Mild athetoid movements were present on examination. Three weeks after presentation she developed recurrent apnoeic episodes associated with generalised slowing on EEG. Her level of consciousness fluctuated. Lactate was 2.5mM/L with an L/P ratio of 31. MRI showed symmetrical hyperintense lesions on T2 in the midbrain and brainstem. Death occurred at 8 months and consent for autopsy was denied.

ALPERS SYNDROME

Case 37 also appeared normal initially but developed intractable myoclonic seizures and regression at 8 months. A female sibling had died following a similar course at 11 months. There were two healthy male siblings and there was no history of consanguinity. Her course was relentlessly progressive with visual loss, increasing weakness, hypotonia and declining level of consciousness. Fronto-temporal atrophy was demonstrated by CT and MRI.The EEG showed slow, high amplitude delta interrupted by bursts of polyspike and wave activity. A severe burst suppression pattern was evident at a later stage. Motor nerve conduction was 43m/second in the median nerve and 26m/second in the lateral popliteal.

She died soon after the onset of liver failure at 10 months. Eosinophilic midzonal necrosis was present in the liver and in the brain neuronal loss and gliosis were confined mainly to the calcerine cortex. These histological findings were compatible with a diagnosis of Alpers syndrome.

Of the remaining 16 patients with encephalopathy, 11 had generalised seizures and 3 of these were resistant to therapy. 12 had developmental regression. Two had delayed milestones from birth. Three had mild generalised weakness and three pyramidal tract signs.

Movement disorders, including ataxia, occurred in 12 children. Eight children had ataxia which tended to occur with exacerbations of their illness. The pyruvate dehydrogenase (PDH) levels were normal in a child with relapsing ataxia as the predominant symptom. Other movement disorders included focal dystonia, bilateral ballismus, athetoid movements and a paroxysmal dystonic disorder.

MYOPATHIC GROUP

Presenting symptoms in the myopathy group were weakness in 4, hypotonia in 2 and ophthalmoplegia in one.

Case 12 presented on day 2 of life with feeding difficulties and tachypnoea. Delivery had been normal. She was found to be profoundly hypotonic with poor respiratory effort, absent reflexes and a weak cry. Marked cardiomegaly was also present. She had a severe mixed respiratory and metabolic acidosis. She was intubated, ventilated and given sodium bicarbonate. Echocardiography revealed hypertrophy of both ventricles without structural malformations. Blood cultures proved negative. Metabolic acidosis failed to improve and sodium bicarbonate was infused in such quantities that she became hypernatraemic. Blood lactate levels were 34 mM/L and pyruvate 550 uM/L. The ratio was 60. At this stage a diagnosis of congenital lactic acidosis was made. Muscle biopsy showed slight accumulation of lipid but no RRF and COX was present. The infant died on day 13.

A brother has myopathic features and there was a paternal uncle who died at one year of age with floppiness and cardiomegaly.

The other children in the myopathic group had much less severe symptomatology. Muscle weakness tended to be mild and proximal, involving limb girdles. Power was usually graded 4/5. Three had mildly myopathic facies.

RRF were present in one (case 10,fig 1), three had increased subsarcolemmal accumulations of mitochondria without RRF and two had EM changes only. None had paracrystalline inclusions but the patient with RRF had rod-like intramitochondrial inclusions. This child had a limb girdle myopathy with marked wasting and prominent scapular winging. He had been floppy since birth and the myopathy was nonprogressive. He had mild developmental delay and markedly reduced ERG responses. Ophthalmoplegia was not present. CK was 350 u/L and lactate 3.9 mM/L. Blood levels of alanine were increased. ECG was normal. Vitamins C and K were of no clinical benefit. A maternal cousin had diabetes mellitus and another had cerebral palsy. Unfortunately these children were unavailable for assessment as they lived some distance away.

Ophthalmoplegia was unusual and there were no cases of CPEO or KSS. One child(case 33) presented with headache and diplopia followed by rapid progression to complete external ophthalmoplegia with unilateral ptosis. Pupils were unaffected but there was some weakness of neck flexion. An edrophonium test, lead levels, and toxin screens were negative. MRI showed increased white matter signal intensity on T2 in both cerebral hemispheres. Lactate and pyruvate levels were elevated and on muscle biopsy there were subsarcolemmal accumulations of abnormal mitochondria. This patient recovered fully within a year and has since been lost to follow up.

Recurrent episodes of weakness of abduction of the eyes associated with rotary nystagmus occurred in one of the other children and remitted spontaneously.

FATAL CASES

There were five deaths. Cases 4,(PDH deficiency), 26, (Leigh syndrome), 37,(Alpers syndrome) and 12(congenital lactic acidosis) have already been mentioned.

The fifth child(case 5) did not have an established diagnosis at the time of death. After thriving for the first 6 months of life he too lost head control and the ability to sit and became increasingly hypertonic. He was admitted with gastroenteritis, hypoglycaemia, metabolic acidosis (pH 7 BE -27 SB

6), ketosis and lactic acidosis (6.8 mM/L). CSF lactate was 3.4 mM/L. CT head was normal. Muscle biopsy showed enlarged abnormal mitochondria. He died following a subsequent similar episode.

THERAPY

No controlled trials of therapy were carried out as presentations were infrequent, sporadic and variable. Carnitine levels were not routinely available but carnitine 100 mg /kg was given to case 5 without improvement. It was discontinued after 2 months and the child subsequently died.

Thiamine, riboflavin, vitamins C and K, biotin and citrosoda were given to individuals on an ad hoc basis. Apart from the apparent benefit of thiamine to patient 36(PDH deficiency), improvement could not be established in any of the others.

BIOCHEMICAL RESULTS

Electrolytes, urea, creatinine, calcium, magnesium and inorganic phosphate were normal in all patients. Blood glucose was low (1.1 mM/L, 1.4 mM/L) on two occasions in case 5. Transaminases were also elevated in this case, as well as in Case 4 who was receiving sodium valproate, (< 2x normal in both), and in the child who died of Alpers syndrome(case 37). The latter also had elevated serum bilirubin and a coagulopathy.

Creatine kinase was elevated in 4 patients (cases 3,10,14,35) with evidence of myopathy. The highest level was 432 U/l (15-130 U/l in males).

Serum lactate was elevated (> 1.8 mM/L) in 34/36 children. The mean was 3.9 mM/L with a standard deviation (SD) of 4. The highest level recorded, 34 mM/L, was in the child with congenital lactic acidosis (case 12). L/P ratios were > 25 in 23 patients and > 35 in 15. The mean was 28 with a SD of 10. Four selected patients had elevation of the lactate/pyruvate ratio confirmed in cultured fibroblasts, (Dr P.T. Owen, C.Path).

CSF lactate was raised in 9 of the 13 patients in whom it was measured.

Serum ammonia was elevated in 9/18 patients, 5 of whom were receiving sodium valproate. In no patient was the level greater than twice normal (19-57 uM/l).

Table 5. Summary of all serum lactate and pyruvate measurements done. AVG is average, STD is standard deviation.

NUMBER	LACTATE mM/l	PYRUVATE uM/l	LACTATE / PYRUVATE	CSF LACTATE mM/l
1	2.40			
1	2.00	78	25	
2	2.90			1.70
3	4.70	114	41	
3	2.00			
3	2.40			
3	3.60	161	22	
3	2.50	120	21	
3	3.30	164	20	
4	3.10	112	27	4.70
4	4.60	156	29	3.40
5	6.80	191	35	
5	4.30	189	23	
6	2.30	112	20	
8	3.40	86	39	
8	1.70	104	16	
7	2.90	78	37	
9	1.50	ND	ND	
10	3.90	442	8	
11	4.60	183	25	.90
11	2.10	130	16	
12	34.00	500	60	
13	2.90	198	14	
13	4.90	129	37	
14	2.70	75	36	
14	3.10	98	31	
15	5.90	110	53	
16	4.10	159	25	
17	2.30	91	25	
17	4.00	180	22	
18	2.00	84	23	1.10
18	2.80	154	18	
19	2.80	69	40	
19	2.10	158	13	
19	6.60	164	40	
20	2.10	94	22	
21	2.70	60	45	
22	2.70	146	18	3.00
23	2.20	98	22	1.80
24	2.80	84	33	2.00
25	10.00			
25	5.30	126	42	
25	5.50	113	48	
26	2.30	102	22	3.40
26	2.50	81	31	
27	3.00	168	18	2.10
28	2.70	108	25	
28	3.20	109	29	
29	2.70	59	46	4.60
30	2.70	132	20	1.50
31	2.70	106	25	4.40
32	2.60	114	23	2.50
32	2.90	110	26	
33	2.40	104	23	
34	1.60	72	22	
35	2.00	54	37	
36	10.60	555	19	
37	3.00	131	23	
AVG	3.92	140.67	28.08	2.65
STD	4.39	97.48	10.82	1.29

Table 5.

Pyruvate Dehydrogenase assay:

19 patients had PDH assays. Two had activities repeatedly lower than controls and one was equivocal. The other 16 assays were normal.

ELECTROPHYSIOLOGY

EEG

Twenty four of 31 patients had abnormalities on EEG. In all, 88 EEGs were performed on the children studied over a period of 9 years. 9 children had only a single EEG and three of these were normal. 6/7 patients with normal EEGs were in the encephalopathy group.

In the muscle group one child had a generalised seizure disorder(case 9). Although she remained seizure free on medication for 18 months, her EEG showed diffuse cortical spiking. Another child with muscle weakness and mild global delay had focal sharp wave activity (case 35) but no seizures. Case 33 had episodic slowing. Three children in this group did not have EEGs recorded and the other had a normal EEG.

Generalised slowing was the most common feature overall, being present in 11 patients. A further 5 had focal slowing. Sharp or spike and wave discharges were superimposed on the background theta or delta frequency in some patients.

Five children had generalised polyspike and wave activity and 8 had focal discharges, usually temporal.

ERG/VER

12 patients had abnormal ERGs with poorly defined, low voltage responses and normal or slightly increased latency. Responses were symmetrical except in one patient. No child had clinical retinopathy and the only one with visual impairment had early optic atrophy and abnormal visual evoked potentials (VEP). The only other child with marked VEP suppression had a normal ERG. He was the patient with MELAS(case 31) and recovered functional vision following his episode of cortical blindness.

BAER

Brainstem auditory evoked responses (BAER) were abnormal in the patient with Leigh Syndrome (case 26). There was increased latency of peaks 3 to 5 with amplitude attenuation.

NCS

Nerve conduction studies (NCS) were performed in 7 children and were normal in 5. The patient with Alpers Syndrome (case 37) had slow, low voltage responses in the lateral popliteal nerve and slowed conduction. The child with PDH deficiency and clinical peripheral sensorimotor neuropathy (case 36) also had abnormal studies.

RADIOLOGY

CT head scan done on 31 patients showed abnormality in 8. Four showed generalised atrophy and one mild frontal atrophy.

Specific findings on the other three were:

1.Putaminal hypolucency (case 32).

2.Extensive attenuation of white matter of internal and external capsules (case 22).

3.Hyperdense lesions in the left internal capsule and globus pallidus (case 36).

MRI

Eleven of the 15 MRI scans done were abnormal. Four had nonspecific changes of ventriculomegaly and prominent sulci in the presence of a normal CT scan. Depending on accessibility and urgency MRI was usually done 2 weeks to several months after CT.

MRI confirmed the basal ganglia lesion found on CT in case 32 and in addition demonstrated a left sided lesion in the cerebellar peduncle. In case 22 extensive T2 hyperintense signals were seen throughout the white matter of the centrum semiovale and in the splenium of the corpus callosum.

Follow up scans showed partial resolution but new lesions were demonstrated in the basal ganglia several months thereafter. There was no accompanying clinical change.

Five children had abnormalities on MRI not identified by CT scan. They were as follows:

1.Extensive T1 hypointense, T2 hyperintense lesions were present symmetrically in the basal ganglia, midbrain, pons and medulla oblongata of the child who died after recurrent apnoeic episodes(case 26). These were thought consistent with a diagnosis of Leigh Syndrome.

2.Low intensity areas in the basal ganglia, external capsule and brainstem in case 29 who presented with unexplained coma and elevated CSF lactate.

3.Prominent ventricular system and cerebellar folia as well as T2 hyperintensity in the paraventricular white matter(case 15).

4.T2 increased signal intensity in the white matter of both cerebral hemispheres in the child with unexplained ophthalmoplegia and no other abnormalities (case 33).

5.A large, left parieto-occipital hypodense lesion in the child with MELAS Syndrome (case 31).

HISTOPATHOLOGY

Succinate dehydrogenase (SDH) and cytochrome c oxidase (cox) stains were not being done by our laboratory at the time of most of these biopsies.

29 patients had a muscle biopsy performed. Results were normal in 2. The patient with MELAS (case 31) did not have a muscle biopsy.

2 had changes of ragged-red fibres. One was a child with proximal myopathy and reduced ERG responses. In addition he had intramitochondrial rod-like particles on EM. The other was a mentally handicapped child with moderate dysmorphic features of hypertelorism, epicanthic folds, flattened occiput and stubby hands. He had partial weakness of the right lateral rectus muscle and an elevated serum lactate (4 mM/l). There did not appear to be any progression of disease during the 4 years he was followed up.

13 biopsies showed focal subsarcolemmal accumulations of mitochondria without the typical "moth-eaten" appearance of ragged-red fibres. These findings were confirmed on EM.

EM was normal or nonspecific in 9 specimens. Abnormalities in the remainder included; enlarged, bizarrely shaped mitochondria with whorled or disorganised cristae, coarse granular aggregates, tubular inclusions and subsarcolemmal accumulations of mitochondria. Increased numbers of mitochondria and lipid accumulations were present in some specimens. These findings were not thought to be artefactual. Paracrystalline inclusions were not a feature.

AUTOPSY FINDINGS

An autopsy was performed on the child with Alpers Syndrome(case 37). In the liver,there was eosinophilic hepatic cell necrosis, mainly in zones two and three, and microvesicular fatty change.

The brain showed non specific degenerative changes in the neurones of the dentate nucleus, hippocampal gyrus and brainstem. The calcarine cortex, which is characteristically singled out in Alpers Syndrome, was particularly affected, with varying degrees of neuronal loss and gliosis.

MITOCHONDRIAL GENETICS

Genetic screening for deletions and known point mutations has recently become available and was not part of the original study. Thus far one patient (case 31) has been identified with the A3243G tRNA mutation and MELAS Syndrome as described.

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DISCUSSION OF FINDINGS

This study approached mitochondrial metabolic derangements from a clinical point of view. It is recognised that there is a broad spectrum of presentations and that certain patients with nuclear encoded mitochondrial defects do not conform to criteria necessary for the diagnosis of MERRF, MELAS or KSS (29). Even patients carrying the mutations responsible for these syndromes do not always present with the classical phenotype. Every effort had to be made therefore to exclude unrelated conditions.

The screening methods available identified not only children with potential oxidative phosphorylation defects, but also those with other mitochondrial enzyme abnormalities such as PDH deficiency. Serum lactate has been used as a screening test for mitochondrial disorders in other paediatric studies. Tulinius et al(59) selected a level of 2.5 mM/L or above in their study of 50 patients. Their laboratory standard values are not reported. Patients in the present study who had a level above the upper limit of the laboratory normal range (1.8 mM/L), were said to have lactate elevation. Venous blood samples were used. Spurious causes of mild elevation such as venous stasis, excessive screaming and struggling in younger children were avoided. Sedation was given before venesection in problematic cases but these were very few. Samples were not taken within 24 hours of a seizure. Patients 19 and 37 may have had elevation due to hepatic dysfunction. Patient 37 had Alpers Syndrome and patient 19 was on sodium valproate. However, both had elevated levels prior to the onset of biochemical liver dysfunction.

The absence of elevated lactate levels cannot be used to exclude mitochondrial pathology. Patients with subclinical or even clinically manifest disorders may have normal serum lactate or fluctuating levels. To detect this group, it may be necessary to take repeated samples, to induce elevation by glucose loading or to do an exercise test.

Glucose loading was introduced by Tulinius in the second half of his study. The maximum lactate level in his confirmed group of mitochondrial encephalomyopathies was 3.0 mM/L after a load of 1.75g/kg glucose. This compared with a mean fasting value of 3.2 mM/l. In the light of these results, the use of this test seems questionable, particularly when the biochemical defect is unknown and glucose loading may have potentially serious effects. Patients with PDH deficiency may respond particularly badly with worsening lactic acidosis.

Exercise testing has not been widely reported. It is not always practical to perform in the ward situation. Petty, Harding and Morgan-Hughes(60) tested 29 adult patients with histologically defined mitochondrial disease. They used standard bicycle ergometry and adjusted the load to produce a heart rate of 150 beats/minute. Samples were taken before and after exercise. The range of lactate values in patients was greater than in controls with some patients reaching a value of 10 mM/L. However, there appeared to be little significant difference in the mean values. Exercise testing was not used in our study as many of the patients were too young or disabled to do the test and for practical reasons it is difficult to standardise.

Histopathological assessment of mitochondrial abnormalities is also not without problems. Adequate sampling of muscle and an experienced pathologist are essential to proper evaluation. Although RRF are the hallmark of these disorders they are not always present nor are they specific(61). Cases without RRF have been reported(61). RRF have also been found in oligosymptomatic relatives (61) of patients with mitochondrial disorders (5/9 in Cialafoni's study). Researchers have found them to be absent in a particular specimen and have identified them in a different muscle(49). Stadhouders demonstrated that RRF are ragged-red only over a limited region of their length(62). As far as possible, clinically involved muscle groups should be sampled, bearing in mind that many patients have subclinical muscle disease. When present in large numbers RRF remain an important indicator of mitochondrial disease.

More common than RRF are ultrastructural abnormalities. These include a variety of nonspecific findings such as mitochondrial enlargement, bizarre morphology, subsarcolemmal clustering and inclusions. The paracrystalline "parking lot" inclusion is regarded as most typical. Accumulations of glycogen and lipid are not infrequently seen. Even when the examiner is satisfied about the absence of artefact it remains difficult to decide whether changes are due to primary or secondary mitochondrial dysfunction.

All the limitations listed above were taken into account in the selection and critical review of cases included in this study.

The 36 patients selected formed a clinically heterogenous group. Major ethnic groups were equally represented. The early age of onset, under 2 years in over 50%, corresponds to that of 0.8 years in Tulinius's study.

Short stature was a common finding, present in 12/36 patients. Abnormalities of growth have frequently been recorded in patients with mitochondrial disease. In Egger's study (63) all 13 patients were short and reduced growth was noted before the third year in all but two, who stopped growing at 5 and 12 years respectively. Growth hormone stimulation tests showed normal results in two and abnormal release in three. Although treatment with growth hormone increased the yearly growth rate, their heights remained below the 3rd centile.
None of our patients had other causes for short stature: limited energy intake was not a factor except in the infant with PDH deficiency who had recurrent vomiting and difficulty in feeding.

Perinatal complications were more common than recorded in other studies (63). In part this may reflect the overall higher incidence of perinatal morbidity in the local population, attributable to late antenatal booking and infrequent clinic attendance, but this is unlikely to be entirely responsible. Metabolic factors seem likely to have played a role in the case of the mother of patient 31, (MELAS syndrome), who herself had developed diabetes and deafness following her first pregnancy. Diabetes alone may cause prematurity. To our knowledge, placental factors have not been studied in mitochondrial disorders. The lack of perinatal complications in the majority of reported cases makes it unlikely that they are a significant factor in mitochondrial diseases.

Although there were three cases with fetal distress, one with asphyxia neonatorum and one with borderline hypoglycaemia on day 2, the subsequent clinical courses of these patients could not be attributed to perinatal problems alone. Patient 20, who had asphyxia neonatorum, was moderately mentally handicapped, as was her sister who suffered no perinatal insult. She had her first seizure at 10 days, later than those usually attributable to hypoxia at birth. The second was at 9 months and ataxia with hyperlactataemia was noted in her third year. The other cases with perinatal complications were not noted to have any abnormalities until after 18 months of age. None has cerebral palsy.

ENCEPHALOPATHY GROUP

MELAS

The patient with MELAS syndrome,(case 31), had a fairly classical course apart from his spastic diplegia. Recurrent stroke-like episodes involving the occipital and parietal lobes are characteristic. These episodes often cause transient cortical blindness, as in our patient, but constructional apraxia and dysphasia have not been widely reported.

The second insult was extensive, yet virtually complete recovery occurred within weeks. There was no evidence of a vascular lesion. Metabolic "strokes" seem to have a cumulative effect with gradual deterioration over time, yet often surprisingly little residua from individual insults. Neuronal death is likely to be far less extensive than that following vascular insufficiency due to other causes.

The exact pathophysiology of these partially reversible lesions remains to be defined. Mitochondrial ultrastructural abnormalities have been identified in endothelial and smooth muscle cells of blood vessels (64) and some authors have suggested that precapillary sphincter dysfunction alters autoregulation, thereby inducing ischaemia (65). However, studies of cerebral blood flow performed during stroke-like episodes have not confirmed this. There has been only a single report of abnormal mitochondria in the neurons of the cerebral cortex (66).

A study of mutant mtDNA content in brain tissue from a MELAS patient found that it was generally in excess of 85%, despite lack of ultrastructural changes, indicating again the inconsistent correlation between ultrastructural and genetic or biochemical abnormalities (53).

The serum lactate of patient 31 was only slightly elevated at 2.7mM/L with that in the CSF being 4.4 mM/L and the lactate/pyruvate (L/P) ratio 25. At this ratio, NADH and NAD are in equilibrium. With respiratory chain dysfunction, an imbalance arises with NADH accumulation driving the reaction towards lactate formation. The ratio may have been higher had it been measured in CSF. Normalisation of levels may occur fairly rapidly following an episode of decompensation. The levels in this case were taken several days after the initial insult. CSF levels may remain elevated longer as they are independent of serum levels and may more accurately reflect cerebral metabolic derangement.

A muscle biopsy was not performed as the clinical features correlated well with the finding of the A3243G mutation and the family were reluctant to consent to further investigations. It would almost certainly have shown RRF which are present in a high proportion of MELAS patients eg. 87% in Goto's (65) study of 40 patients.

Determination of specific biochemical defects was not possible. Complex I deficiency is most commonly described in association with MELAS, but complex IV and combined defects also occur. Some patients with documented clinical and genetic features of MELAS show no abnormality on currently available biochemical tests of the respiratory chain in muscle. Perhaps these would be demonstrated were it possible to test neural tissue.

The mother of this patient is almost certainly a carrier of the MELAS mutation. Diabetes and deafness have now been reported in maternal relatives of many MELAS patients. It has as yet not been possible to document the extent of the carrier status in this family as members remain unwilling to be tested.

Successful treatment of MELAS with vitamins and coenzymes has been reported, but efficacy has been difficult to document. Ihara (66) reported successful treatment of a 64 year old woman with MELAS. Her ataxia and muscle weakness improved after treatment with coenzyme Q and idebenone. EEG showed disappearance of slow wave bursts and dementia also improved. Improvement in nerve conduction velocity was documented. However, she had not had any stroke-like episodes for several years prior to treatment. Other authors (67) also report improvement in patients with complex I deficiency when treated with riboflavin. Therapy appeared to benefit those with myopathy more than those with encephalopathy. There is a report of a single patient with MELAS (68) whose previously frequent stroke-like episodes (8 admissions/18 months) disappeared for 18 months on treatment with nicotinamide and riboflavin. Stroke recurred 7 days after cessation of therapy.

Other researchers have found no benefit from this treatment. Mathews(69) studied 16 patients with a variety of mitochondrial disorders over one to four 2 month periods of treatment with coenzyme Q, vitamins K and C, riboflavin, thiamine and niacin. Response to treatment was monitored by exercise testing and phosphorus magnetic resonance spectroscopy (MRS) in resting and exercised muscle. No clear improvement could be documented.

In view of individual cases with reported improvement on vitamin therapy and the lack of any alternative treatment, it is probably best to assess the clinical response in each patient. Where biochemical diagnosis is not available this will remain a process of trial and error but significant side effects need not be expected.

MELAS was considered a possible diagnosis in four other patients (cases 23,29,22,32) with stroke-like episodes.

Patient 23 suffered recurrent stroke-like episodes from the age of 2 years. These caused progressive spastic quadriparesis, aphasia, ataxia, internuclear ophthalmoplegia and pseudobulbar palsy. No vascular cause was identified. CT and MRI scans were repeatedly normal and pathology was assumed to involve midbrain and brainstem rather than cortex. Intellectual function was largely spared as was somatic growth. In Goto's study (65) of 40 MELAS patients, 62% of those with the 3243 mutation were short and 72% developed mental handicap.

There was some evidence to support a diagnosis of mitochondrial dysfunction in that lactate was elevated in serum and at the upper limit of normal in CSF. In addition biopsy showed enlarged mitochondria with abnormal cristae and some subsarcolemmal clustering. This is significant because muscle abnormalities, other than disuse atrophy, are not usually found in CNS disorders.

On the other hand, the absence of cortical infarcts, dementia, stunted growth and RRF makes MELAS an unlikely diagnosis in this case. All that can be said is that there is some evidence of mitochondrial dysfunction and a lack of any alternative plausible diagnosis. Some comparisons could be drawn between this case and cases of alternating hemiplegia of childhood in which muscle mitochondrial dysfunction has recently been demonstrated (70).

Three other patients (cases 29,22,32), have had single stroke-like episodes only. Follow up has been between 2 and 4 years.

Patient 29 had a number of features suggestive of a mitochondrial disorder. She experienced an unexplained episode of weakness followed by depressed level of consciousness. EEG showed generalised slowing and MRI showed low density areas suggestive of infarcts in the basal ganglia, external capsule and brainstem. These were not as symmetrical as the lesions seen in classical Leigh syndrome. CSF lactate was considerably elevated at 4.7 mM /L as was the L/P ratio, at 46. No other explanation for the lactate elevation or the significantly increased ratio could be found. The involvement of the basal ganglia was also suggestive of a metabolic disorder. The clinical features could have been caused by encephalitis, but there was no fever, leucocytosis or CSF abnormality to substantiate this. Toxin ingestion was also a possible explanation but there was no history of this and screening tests were negative.

She recovered over 4 weeks but has residual moderate mental handicap. There has been no progression in the follow up period. A mitochondrial disorder is considered highly likely in this patient. A transient disturbance of respiratory chain function caused by an unknown toxin is the most probable alternative explanation.

Patient 22 developed progressive quadriparesis with CT and MRI evidence of extensive demyelination. CSF lactate was elevated. Clinical recovery from initial severe disability occurred with mild residual spasticity. There have been no subsequent clinical episodes. The clinical picture was not that of one of the degenerative leukodystrophies. Metachromatic and adrenal leukodystrophy were excluded biochemically. A post infectious demyelination was considered as there had been a recent upper respiratory tract infection, but this did not explain the elevated CSF lactate or the appearance of new lesions in the basal ganglia several months after the initial insult. There was no elevation in CSF protein or cell count. In parainfectious disorders the cerebellum and brainstem are more often involved than cortical white matter. Multiple cortical lesions have been described (71) but none as extensive or confluent as in our patient. Acute disseminated encephalomyelitis is usually accompanied by seizures and alteration in level of consciousness. Neither were features in this case.

One of the childhood forms of multiple sclerosis was included in the differential diagnosis. These are extremely rare in children under 10 years. Schilder disease would be the form most likely in the presence of normal CSF protein and confluent plaques on imaging. However in this instance the lesions did not enhance with contrast as described in Schilder disease. Abnormalities in lactate metabolism have not been described in this disorder.

A recessive familial leukoencephalopathy is another possible diagnosis but there was no history of consanguinity to support this. It thus seems most feasible that an occult metabolic error became apparent under conditions of increased stress, provided perhaps by a viral infection.

Extensive demyelination is not a characteristic finding in mitochondrial disorders. It is unusual in MELAS and when present in KSS and MERRF, occurs in conjunction with areas of spongy degeneration (53). There has been a recent report of a set of dizygotic twins with non-progressive leukoencephalopathy and large , confluent T2 hyperintense lesions on MRI (72). One twin had myopathy and the other myopathy with myoclonic

epilepsy. The 3243 MELAS mutation was detected in the latter, at lower levels than occur in patients with MELAS. The mutation could not definitely be linked to the demyelination or the muscle pathology.

Patient 32 with focal dystonia also provided diagnostic difficulties. The clinical course and CT findings were not those of classical torsion dystonia. There were not the obvious diurnal fluctuations of Segawa disease. Other causes of putaminal hypolucency including arterial infarcts and viral infection could not be excluded but there was no evidence to support their presence.

Examination of the patient's retina showed hyperaemia, some tortuosity and dilatation of vessels and some haemorrhages. Visual acuity was 6/9. Similar findings have been described in patients with Leber's Hereditary Optic Neuropathy (LHON). Some families with LHON have been reported with a hereditary spastic dystonia and putaminal necrosis (73). Onset was between 5 and 9 years with normal early development and a stationary or slow progressive course. The dystonia in these patients was associated with spasticity and often involved facial and bulbar muscles with dysarthria. Bilateral or unilateral putaminal hypodensity was present. This disorder has been described in only three families. This patient has no family history of blindness but this unusual form of LHON nevertheless deserves consideration in the differential diagnosis.

PDH DEFICIENCY

Two patients (cases 4, 36) in this study had documented PDH deficiency. Neither had a positive family history. The majority of documented defects of PDH involve the E1 alpha subunit which is coded by DNA on the X chromosome. This E1 component is dependant on a thiamine pyrophosphate cofactor. A predominance of males has been reported in some studies but there have also been many females affected to varying degrees. The explanation for this could be that the pattern of X inactivation within tissues leads to variable expression, or alternatively that the defect lies in one of the other subunits of PDH. The E1 beta and E3 components have been mapped to chromosomes 3 and 7 respectively (74).

Skin fibroblast activities of PDH do not always correlate with the clinical severity. The degree of activity varies in different tissues so that fibroblast levels may not be representative of brain PDH activity. This cannot be explained by heteroplasmy as PDH is nuclear encoded. It is possible that different tissues recognise and degrade mutant proteins variably, but this remains to be proved.

The specific defects in these two patients could not be defined, but pyruvate decarboxylase(E1) deficiency is probably the most likely. The second case, 36, was similar to one described by Evans (75), which also showed episodic weakness, ataxia and impaired ocular motility of acute onset following an infection. Recovery started after 2 weeks and took 3 months. Marked growth retardation was present. Unusual was that patient 36 subsequently thrived and made a remarkably rapid recovery from a very disabled state. Although it seems reasonable to attribute this at least in part to thiamine, this could not be substantiated biochemically.

The ERG findings in this patient are also unusual. They are reproducible and are too marked to be artefactual. No visual impairment is evident nor any clinical retinopathy. The possibility of dual pathology has been considered but the presence of a second rare inherited disorder seems unlikely. Screening for mitochondrial deletions has been negative.

He will be maintained on thiamine and a high fat low carbohydrate diet.

LEIGH SYNDROME

The patient with Leigh Syndrome had a typical course with normal early development, followed by regression, seizures, choreoathetoid movements and respiratory abnormalities. MRI showed symmetrical T2 hyperintense lesions in the midbrain, pons and medulla. PDH deficiency was not excluded in this patient. Complex I and cytochrome c oxidase deficiency have also been documented in Leigh syndrome but the resulting phenotypes are indistinguishable. The COX defect would appear different to that documented in MELAS in that inheritance follows a recessive pattern, indicating a nuclear defect rather than one in mitochondrial DNA (76).

There is a recent report of an 18 year old patient with features of MERRF but without RRF, who developed Leigh syndrome, confirmed at autopsy (77). The 8344 mutation, characteristic of MERRF, was found in high percentages: blood: 81%; skeletal muscle: 99%, ocular muscle:97%, cerebellum:97%, cerebral cortex: 97%, heart: 97%, liver:99% and kidney: 98%. The patient's normal mother had 72% in blood and probably more in other tissues. This case demonstrates a number of interesting features:

a) the correlation of the 8344 MERRF mutation with Leigh Syndrome.

b) the absence of RRF in a patient with features of MERRF and a very high proportion of mutant DNA in muscle. Severe cytochrome c oxidase deficiency was documented histochemically and there was evidence of increased mitochondrial mass. Other patients reported with MERRF have had only 50 or 60 % mutant mtDNA in muscle. This suggests that factors other than mitochondrial proliferation must influence the development of RRF.

c) the absence of liver pathology in the presence of 99% mutated mtDNA. It is to be expected that these levels would exceed the threshold for disease expression and again as yet unknown factors must play a role.

Patients with Leigh syndrome have also been found to carry the NARP mutation. There is thus increasing evidence that the pathological features of Leigh Syndrome may coexist with severe phenotypic expression of several different mtDNA mutations(80).

ALPERS SYNDROME

A recessive pattern of inheritance was likely in the child who died of Alpers Syndrome (case 37). A sibling had died of a similar illness. There was no known consanguinity and no relatives were affected. The biochemical and genetic basis of Alpers Syndrome is unknown but some authors have recently suggested a mitochondrial aetiology (78). It may become evident that different mutations are responsible for a similar phenotype, as in Leigh Syndrome.

The clinical course of the patient was very typical with initial normal development followed by failure to thrive, intractable seizures, regression, and ultimately liver failure.

Eosinophilic midzonal hepatic necrosis was present as reported (43). Valproate therapy, which was used in this case, has divided opinion on the aetiology of liver damage (43). In Harding's series of 32 patients (43), probably the largest group reviewed, valproate therapy appeared unrelated to outcome. Two pairs of siblings in this study exhibited identical hepatic pathology in the presence and absence of the drug.

Selective involvement of the calcarine cortex, as documented at autopsy in patient 37, is characteristic of Alpers syndrome (43). It is generally accepted that cerebral damage in these cases is not solely as a result of uncontrolled seizure activity or hepatotoxicity (43). The aetiology of this form of hepatocerebral degeneration remains to be elucidated.

MERRF-LIKE SYNDROME

Patients 1, 3 and 28 had many of the features of MERRF syndrome but without RRF. Patient 3, in particular, had progressive myoclonic epilepsy, ataxia, weakness, dementia short stature and pyramidal tract signs.

Myoclonus is often the presenting feature in MERRF. It tends to be stimulus induced and may occur with or independently of ictal discharges. It may be difficult to control. The three patients in this study had mild myoclonus which was not stimulus induced; it was frequently but not always associated with ictal episodes and could be controlled with benzodiazepines. Ataxia worsened during episodes of seizure exacerbation. Anticonvulsant toxicity was not found to be responsible. Hyperlactataemia was consistently present in all three patients, independant of seizure activity. L/P ratios were elevated, suggestive of respiratory chain dysfunction.

Neither patient 1 nor patient 3 had RRF. Cases of MERRF who carry the 8344 mutation but who lack RRF have been described. Graf and coworkers (57) reported a mother and her two symptomatic children in whom only the mother had RRF, despite a higher proportion of mutant DNA in the children's biopsies. Findings such as these have yet to be explained. A possible explanation may be found in the role played by mutations induced by aging.

Silvestri found a high correlation between the clinical phenotype of MERRF and the 8344 mutation when screening 150 patients with suspected mitochondrial encephalomyopathy (24). Sixteen of 150 patients had the mutation. Eleven had MERRF, 2 had other mitochondrial encephalopathies, 2 had limb girdle myopathy and 1 had PEO. This would indicate that if only limited numbers can be screened, those patients with the characteristic phenotype and RRF are most likely to be positive.

Despite the absence of RRF, patient 1 had significant abnormalities on EM of biopsied muscle.

There was insufficient evidence to identify these patients positively as having MERRF syndrome although they had a number of characteristic features. They may belong to a nuclear encoded subgroup of mitochondrial myopathies but biochemical confirmation of respiratory chain derangement is needed to clarify this diagnosis.

Two patients had overlap syndromes with features of MERRF and MELAS:

Patient 22 had ataxia, optic atrophy, ophthalmoplegia and an intractable seizure disorder with myoclonus and stroke-like episodes. He had one admission with severe lactic acidosis. The features were highly suggestive of mitochondrial disease. His parents were first cousins indicating that he may have a nuclear encoded defect. Juvenile neuronal ceroid lipofuscinosis (NCS) was considered but this could not explain the lactic acidosis and there was no histological evidence to support this diagnosis. In addition he has had periods of remission from seizures and remains ambulant 7 years after the onset of symptoms.

Patient 19 had normal early development followed by atonic attacks, mental deterioration and an episode of unexplained coma. Features in favour of mitochondrial disease were lactic acidosis and an elevated L/P ratio. Lipid accumulation was present on biopsy. There was mild elevation of transaminases but no hypoglycaemia. He had been receiving valproate therapy for almost 4 years before his admission with reduced level of consciousness. It seems unlikely that his state could have been due to toxicity, but the drug was nevertheless discontinued. CT scan showed no specific features and MRI was not performed. Lesions may have been identifiable had this been done but there were no focal signs and he recovered to his former level of function.

The remainder of the encephalopathy group had nonspecific findings. Seizure disorders and developmental regression were the most common. Generalised seizures were most frequent.

Movement disorders occurred in four patients. The child with ballismus (case 11) had prolonged depression in consciousness following a first episode of seizures. She was from a country area and may have become hypoxic during status epilepticus. PDH activity was normal. Ballismus resolved but she suffered residual moderate mental handicap.

There was no adequate explanation for the clinical signs, lactate elevation and EM changes found in the other patients. Mitochondrial disease could neither be confirmed nor excluded.

MYOPATHY GROUP

The infant with congenital lactic acidosis (case 12) almost certainly had mitochondrial dysfunction. The presence of cardiomyopathy is not typical of PDH deficiency or fatal infantile lactic acidosis caused by cytochrome c oxidase deficiency as described by DiMauro (79). Both benign and fatal infantile mitochondrial myopathy show COX negative fibres which this patient did not have. Defective NADH oxidation has been demonstrated in an infant with a similar disorder but without cardiomyopathy (80).

The family history suggests that there may have been an affected paternal uncle. This reduces the likelihood of the maternally inherited myopathy and cardiomyopathy which has been described (81). Dilated cardiomyopathy has been described with multiple deletions (82). The defect in our patient would appear to be nuclear encoded.

The clinical features of patient 10 were compatible with a diagnosis of congenital myopathy except that he had subclinical retinopathy. The histological and biochemical features suggest a mitochondrial abnormality with RRF and hyperlactataemia, 3.9 mM/L. Histological features of other congenital myopathies were not present. The rod-like inclusions were intramitochondrial.

Mitochondrial myopathies seldom present in the neonatal period and when they do, are usually indicative of a severe defect. However, forms of mitochondrial myopathy which undergo spontaneous improvement have been reported and in general there is such a degree of phenotypic heterogeneity that mitochondrial myopathy remains the most acceptable diagnosis in this patient.

Patient 33 had a relatively acute onset of progressive ophthalmoplegia involving all external ocular muscles. This resolved over several months. Lactate was 2.4 mM/L. Initially he was thought to have extensive RRF but review of the biopsy showed subsarcolemmal mitochondrial accumulations only. A variant of the Miller Fisher syndrome was considered but there was no ataxia, reflexes were normal and CSF protein was not elevated. Areas of demyelination in the cerebral hemisphere are not a feature of Miller Fisher syndrome.

There was no history of snake bite. Some local varieties of snake venom cause cranial nerve paralysis.

A mitochondrial disorder could not be excluded as there was some evidence in the muscle biopsy to support this diagnosis. Recurrent episodes of ophthalmoplegia may occur with PDH deficiency but there are usually other signs. This patient was lost to follow up. It is to be expected that he would have returned to the hospital had he suffered further episodes. The explanation for his symptoms remains unknown.

FATAL CASES

No diagnosis could be made in patient 5 who died. The marked lactic acidosis was in keeping with mitochondrial disturbance. The presence of large quantities of ketones reduced the likelihood of carnitine deficiency. Recurrent episodes of decompensation occurred so an extraneous toxic cause was also unlikely.

BIOCHEMISTRY

The mean lactate level in these patients was greater than twice the upper limit of normal and was above Tulinius' mean of 3.0 mM/L. There was a large SD indicating wide scatter. Only 2 patients had levels lower than 1.8 mM/L; these were 1.5 and 1.6 mM/L. Neither had the test repeated or was stressed.

The mean L/P ratio was also elevated with a large SD. Values ranged from 8, in patient 10 with a lactate of 3.9 mM/L and pyruvate of .442 mM/L, to 60 in the infant with congenital lactic acidosis (case 12).

CSF lactate was elevated in 70% of patients in whom it was measured. This is significant as CSF lactate is metabolised independently of serum lactate and probably better reflects cerebral metabolism.

These results are useful as a guide but cannot be considered in isolation, as discussed. The mean values would be consistent with defective oxidative phosphorylation.

ELECTROPHYSIOLOGY

The generalised slowing on EEG found in 35% of patients is characteristic of metabolic dysfunction. One was a postictal record and is not included. Focal and generalised epileptic discharges were common. Tulinius documented similar findings in his patients but to our knowledge there has not been detailed reporting of EEG changes in paediatric patients with mitochondrial pathology.

A careful study in 13 adult MERRF patients showed slowing in 85% with bursts of synchronous frontal delta activity in 10/13 (83). 70% had bursts of spike and wave or polyspike and wave activity and 6 had focal discharges. Action and intention myoclonus were not clearly related to EEG discharges.

Twelve of our patients had abnormal electroretinograms without clinical evidence of retinopathy. Technical error was excluded as far as possible. In most reported series, ERG has been done when there is already evidence of retinopathy. Its usefulness as a screening test has not been established, but it is reasonable to assume that it may be a more sensitive indicator of dysfunction than the presence of overt retinal changes. These cases will be followed to monitor any progression.

RADIOLOGY

Relevant findings have been discussed in conjunction with case histories. MRI was more sensitive than CT scan particularly when visualising basal ganglia, midbrain and brainstem structures. A direct comparison could not be made owing to the time lapse between scans.

Specific imaging features have been described in MELAS and Leigh syndrome but more than 50% of MERRF patients and many of those with KSS have normal imaging or nonspecific changes (84).

HISTOPATHOLOGY

Only two of the patients studied had RRF. More might have been expected in a study of patients with mitochondrial disease. The succinate dehydrogenase stain (SDH), a more sensitive marker of mitochondrial proliferation than the Gomori, was not used routinely. Only two of the 29 patients who were studied had normal biopsies; the rest showed subsarcolemmal accumulations of mitochondria or mitochondrial ultrastructural changes. It cannot be proved that these arose as a result of primary mitochondrial dysfunction, but such a high incidence of skeletal

muscle abnormalities would not be anticipated in a group of children with mainly CNS pathology. Hypoxia could be documented as a possible contributing factor in one child only. No extraneous toxins were identified.

In Tulinius's study, 17/20 children with confirmed mitochondrial encephalomyopathy had ultrastructural changes in mitochondria at biopsy. Only 7 showed typical RRF. Two had MERRF, two had MELAS and two had KSS. The other child had features of MERRF but without myoclonus. Three children with normal muscle biopsies had complex I deficiency. This illustrates that although there is a good correlation between RRF and the classical MERRF, MELAS, KSS phenotypes there may well be a larger group of patients with as yet unidentified genetic defects or partial expression of known defects who do not have RRF.

CONCLUSIONS

This study was undertaken to provide a comprehensive data base on patients with suspected mitochondrial disease seen at the Red Cross Childrens' Hospital. Only children with neuromuscular features were examined and there may well be others whose presenting complaints prompted referral to the cardiology or endocrine clinics.

The study identified 30 patients with an encephalopathic clinical picture and 7 with a myopathic picture. A mitochondrial disorder was positively identified in 5 cases: MELAS syndrome (case 31), PDH deficiency (cases 4,36), Leigh syndrome (case 26) and Alpers syndrome (case 37). In the remainder, clinical, biochemical and histological data available were suggestive of mitochondrial pathology but this could not be confirmed. As the features in many cases were not typical of MERRF, MELAS or KSS and clear matrilineal inheritance was not evident, it is likely that some of these children fall into DiMauro's mitochondrial encephalomyopathy or myopathy groups with complex I or III defects. These are nuclear encoded and the genes have not been identified. They manifest with a broad spectrum of clinical features similar to those found in our patients; dementia, seizures, ataxia, retinopathy, myopathy and movement disorders.

An impression was gained that mitochondrial disorders may be underdiagnosed at the hospital. Ability to confirm this impression was limited by the lack of specialised investigations. In order to investigate selected patients further, biochemical assay of respiratory chain function is necessary. This would provide a better idea of the prevalence of these disorders. Such a screen would need to be specific, accessible and

inexpensive. The feasibility of measuring ATP production in permeabalised fibroblasts by a fluorometric method is currently being investigated by the author.

Establishment of an assay to measure total, free and esterified carnitine in serum and muscle would be of value in excluding disorders due to primary carnitine metabolic problems and in diagnosing secondary carnitine deficiency caused by respiratory chain dysfunction (85) or valproate therapy.

There are many neurological disorders that remain as yet unexplained in terms of their genetic and biochemical aetiology. The discovery of the "25th chromosome", mtDNA, has provided researchers and clinicians with some insight into the mechanisms underlying a small group of these diseases. Since genetic screening for deletions and point mutations in mtDNA can now be performed locally, the diagnosis can be confirmed in certain cases.

Much of the challenge still remains. As appropriate treatment in any medical problem relies on an accurate diagnosis, it is hoped that further research will not only aid the clinician in making the correct diagnosis but will pave the way for preventive and curative therapy.

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