

Monozygotic twins with MELAS-like syndrome lacking ragged red fibers and lactacidaemia

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Typical cases of MELAS present a combination of clinical and neuroradiological features, lactacidaemia, and ragged red fibers (RRFs) in striated muscle. We have observed a MELAS-like syndrome in monozygotic twins. They developed seizures typically in conjunction with physical exertion, sleep deprivation or febrile episodes. Stroke-like episodes occurred usually during seizures. In twin 2 the course was fatal at age 20 years. Neuroradiological findings were typical of MELAS. Plasma lactate was normal in both. CSF lactate was normal in twin 1 and normal/elevated in twin 2. RRFs were not seen in muscle biopsies of the twins. Complex I activity was reduced in muscle in twin 1. Brain tissue removed at epilepsy surgery in twin 2 showed the presence of mitochondrial angiopathy. The commonest mitochondrial DNA mutation in MELAS, at base pair 3243, was absent. Lactacidaemia and mitochondrial myopathy with RRFs constitute part of the diagnostic criteria of MELAS. However, the absence of these features does not exclude mitochondrial disorder with the serious manifestations of MELAS (seizures and stroke-like episodes) as seen in these twins.

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Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is a distinct syndrome with ragged red fibres (RRFs) in muscle biopsy, normal early development, short stature, seizures, hemiparesis, hemianopsia or cortical blindness. Lactacidaemia is a common finding (1). Complex I deficiency is the commonest biochemical defect (2). Multiple defects of respiratory chain enzymes have also been reported (3, 4). An A3243G mutation of the mitochondrial DNA (mtDNA) tRNA^{Leu(UUR)} gene was demonstrated (5, 6) and detected in 80–90% of MELAS patients (4). Other mtDNA mutations in MELAS are T3271C (7) and T3291C (8). The A3243G mutation is not specific for MELAS and associated with a variety of other phenotypes as well, such as progressive external ophthalmoplegia (PEO), Kearns–Sayre syndrome (KSS) (9), deafness and diabetes mellitus (10–12), and myoclonic epilepsy with RRF (MERRF) (13).

This report deals with a MELAS-like syndrome in monozygotic twins. They developed focal status epilepticus (FSE) – epilepsy partialis continua (EPC), generalised seizures, and neurological deficits occurring usually during periods of seizures. Ptosis was present in both, but no limb muscle weakness. One of several diagnostic possibilities of FSE-EPC is mitochondrial syndromes (14).

Subjects and methods

There was no family history of epilepsy, strokes or muscle disease. The parents were non-consanguineous, healthy, and normal on neurological examination. The mother had hypertension during the first pregnancy. A daughter was born in 1967, who was healthy and clinically normal at age 26. She was the shortest in the family, 156 cm. The second pregnancy was complicated with Rhesus immunisation. Labour was oxytocin induced at term. Male twins with one placenta were born in 1973.

Case reports

Twin 1 – The APGAR score was 9 after 1 minute. Birth weight was 2240 g. Due to anaemia and haemolysis a blood exchange was performed. Sucking was poor and naso-gastric feeding was necessary for a period. He had eczema, infections, poor weight gain, and was repeatedly hospitalised the first 2 years. Weight was 5.8 kg at age 1 year. He could sit without support at 16 months and walk at 26 months. He was late to talk. After age 2.5 years he developed normally.

He did not participate in sports. At age 12 he became ill with vomiting, headache, and fever. In the course of 12 h he had repeated focal seizures. The electroencephalogram (EEG) showed signs of FSE of the right cerebral hemisphere. Herpes- and cytomegalovirus serology were negative. Pheno-barbitone was given and he appeared to recover completely. At age 15 a generalised seizure occurred at night. On admission to hospital neurological examination was normal.

At age 16 repeated focal seizures of the left arm and generalised tonic-clonic seizures occurred. FSE-EPC continued for several days and the left arm was paretic. Non-contrast enhanced computed tomography (CT) was normal. Later that year, after sleep deprivation and physical exertion, he had episodes of repeated focal seizures of the right arm and neck. Initially he was febrile. EEG showed FSE of the left hemisphere. Weakness of the right arm and ptosis persisted for several days. He had rightsided hemianopsia, difficulties to read and write, and coordination problems. Non-contrast enhanced CT showed a relatively large area of low attenuation in both white and gray matter of the left occipital and parietal region and a small low attenuating lesion in the left thalamus.

Between ages 17 and 21 ys he had several episodes of headache and cortical blindness. Vision improved during the course of 24 hours. Visual acuity was 0.2 at the worst. Two of the episodes were accompanied by an epileptic seizure. He experienced visual deterioration in conjunction with physical exhaustion and headache. Vision improved on resting. At age 19 CT with contrast enhancement showed a smaller leftsided occipitoparietal low density area with local widening of the sulci and enlargement of the posterior horn. Magnetic resonance imaging (MRI) showed a similar high signal lesion on T2-weighted images. Contrast enhancement was not seen. A later MRI that year showed a new high signal lesion in the right occipital region. The leftsided occipital lesion was unaltered. Vertebral angiography did not show any abnormalities. At age 20 visual acuity was 0.8 on the right and 1.0 on the left. The hemianopsia

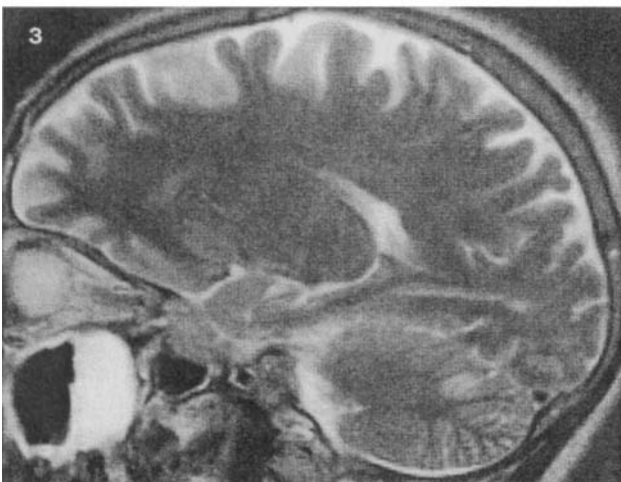
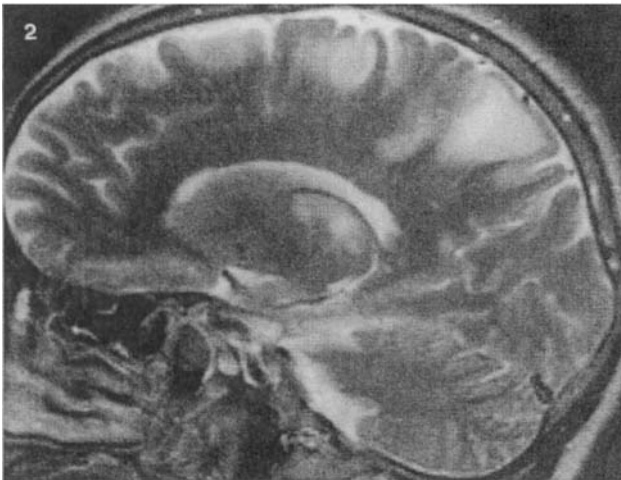
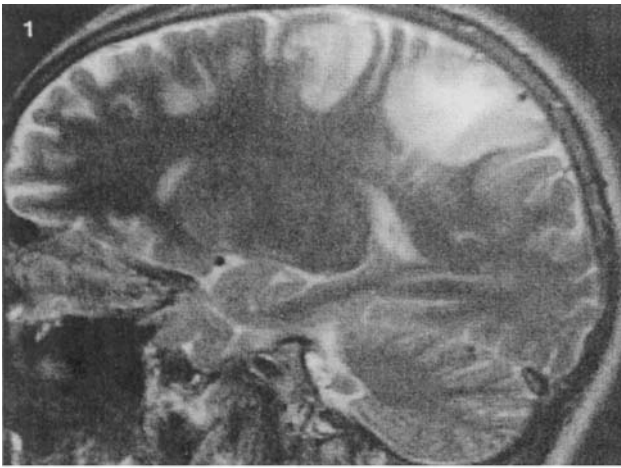
contracted 4 years earlier had resolved considerably. Visual perception was decreased but has improved further in recent years. Achilles tendon reflexes were absent.

The levels of plasma (p)- lactate at rest, measured on seven occasions, ranged between 1.1 and 1.7 mM (normal 0.8–2.0 mM). Cerebrospinal fluid (CSF) lactate levels, measured on three occasions, were 1.9–2.0 mM (normal <2.3 mM). These investigations were performed between ages 17 and 19 ys. Urinary (u)-organic acids were normal. P-amino acids, serum (s)-ammonia, creatine kinase (CK), and bilirubin levels were all normal. Transaminases were somewhat elevated considered due to anti-epileptic treatment. The electrocardiogram (ECG) showed right incomplete bundle branch block. Echocardiography was normal.

Twin 2 – The APGAR score was 5 and 7 after 1 and 5 minutes. Birth weight was 2190 g. A blood exchange was performed due to haemolysis. Ultra-violet radiation was given. Initially he was nourished with a naso-gastric tube as sucking was poor. He had childhood eczema. Weight was 8 kg at age 1 year. He could sit and walk at an earlier age than his brother.

Unlike his brother he took part in sports. He was healthy until age 19. Then, after strenuous physical activity, partying (sleep deprivation), followed by physical exertion, a tonic seizure with postictal confusion occurred. Nothing abnormal was noted on clinical examination. EEG showed bilateral synchronous epileptogenic activity with right sided predominance. MRI was normal. Carbamazepine treatment was instituted. On neurological examination 6 months later fine motor skills and balance were decreased, the tendon reflexes of the left arm were increased and the achilles tendon reflexes weak.

At age 20 yrs, again after sleep deprivation (partying) and physical exertion, left sided FSE/EPC commenced, and episodes of generalised tonic-clonic seizures occurred. From the first day there was a central weakness of the left arm and positive Babinski on the left. Two days after hospital admission he was febrile. CSF contained no cells. EEG showed FSE of the right hemisphere. MRI four days later showed increased signal intensity on T2-weighted and low signal on T1-weighted images parietally, and anterior to the central sulcus, as well as in the thalamus on the right side. Similar changes were present in the left superior cerebellum. Early during the course seizures were intermittent. The longest seizure-free intervals were 24 h. Seizures reappeared a few hours after mobilisation. MRI (Figs. 1–3) 14 days after the onset showed regression of the lower parietal changes. There was no



Figs. 1–3. High signal lesions in T2-weighted MR images in twin 2. Cortex and subcortical white matter are affected in the parietal and posterior frontal lobes (Figs. 1, 2). Additional high signal lesions in the thalamus (Fig. 2) and contralateral cerebellar hemisphere (Fig. 3).

contrast enhancement. MRI four weeks after the onset showed some progression in the parietal and frontal changes on T1-weighted images. Partial regression of the left cerebellar lesion had occurred. Clonic twitches were more or less continuous in the left hand. There was action myoclonus. At times the left arm, neck, face, chest, and stomach were jerking. Palatal myoclonus and rhythmic activity of the eyelids occurred. Bilateral ptosis developed. No signs of hemianopsia were found. The left arm remained paretic with increased tendon reflexes. Later the left leg became weak. There was ataxia and absent vibration sense in the toes.

Medication included various combinations of phenytoin, carbamazepine, lignocaine hydrochloride, betamethasone, lamotrigin, and intravenous infusion of diazepam (up to 20 mg/h). Carnitine and B vitamins (riboflavin, thiamin, pyridoxine) were also administered. The seizures were not aborted. He was then treated in a ventilator. Repeated thiopentone coma treatments were given. Propofol was also tried. Seizures continued as soon as thiopentone/propofol administration was reduced. Serial EEGs at most instances showed continuous epileptogenic activity of the right hemisphere. A decision on surgical treatment was made. Multiple subpial transections of the central region of motor and sensory cortex of the right hemisphere were performed. The total amount of focal seizures decreased but the generalised seizures became more frequent. A week later a large frontal lobe resection was made. Generalised seizures continued. He developed bilateral occipital infarcts and diffuse, probably ischaemic changes in the remaining part of the right frontal lobe. Seizures now appeared regularly in the right extremities and subsided on the left. The last three months of his life he was treated with phenytoin and intravenous midazolam 17–20 mg/hour. Myoclonus and seizures continued and worsened on slowing the infusion. Terminally he was tetraplegic. Brain stem reflexes were retained. Five months after the onset of FSE-EPC he died.

The laboratory tests were performed after onset of seizures at age 20. The levels of p-lactate were 1.2, 1.8, 1.5, and 2.0 mM. CSF lactate levels were 2.2, 3.2, and 9 mM (the latter when in thiopentone coma and without epileptogenic EEG activity). S-carnitine, CK, myoglobin, transaminases, lactate dehydrogenase, alkaline phosphatase, bilirubin, homocysteine, complement, and antinuclear antibody were all normal. U-organic acids, carnitine, and amino acids were also normal. ECG showed non-specific T-wave flattening fronto-laterally.

Muscle biopsies

Muscle biopsies were obtained from the tibialis anterior in twin 1 at age 19 ys and from the vastus lateralis of the quadriceps muscle in twin 2 at age 20 ys. The biopsies were instantly frozen and cryostat sections prepared. The staining methods included haematoxylin-eosin (HE), the myofibrillary ATP-ase reaction at pH 9.4 and 4.6, modified Gomori's trichrome, oil red O, periodic acid Schiff (PAS), NADH-tetrazolium reductase (NADH-TR), succinate dehydrogenase (SDH), and cytochrome c oxidase (COX). Other pieces were fixed in buffered glutaraldehyde, postfixed in osmium tetroxide, and epon-embedded for electron microscopy (EM).

Biochemical analysis in muscle

One part of the muscle biopsy from each twin was used for determination of mitochondrial ATP production and enzyme activities. The control group used for these determinations consisted of 11 healthy subjects (age 33 ± 10 years, mean \pm SD), all of whom led a sedentary life without regular physical exercise. The rate of ATP production in mitochondria isolated from fresh muscle was determined as described previously (15). Eight different substrate combinations were used to test the mitochondrial function: a) pyruvate+palmitoyl-L-carnitine+ α -ketoglutarate+malate (PPKM), b) glutamate+malate (Glu+Mal), c) N,N,N¹,N¹-tetramethyl-1,4-phenyldiamine+ascorbate (TMPD+Asc), d) α -keto-glutarate, e) palmitoyl-L-carnitine+malate (PalCar+Mal), f) pyruvate+malate (Pyr+Mal), g) succinate+rotenone (Succ+Rot), and h) succinate (Succ) only.

The activities of the enzymes in the respiratory chain were determined spectrophotometrically in homogenates of fresh muscle at 25°C. COX and succinate-cytochrome c reductase (SCR) were determined (16, 17), and rotenone sensitive NADH-cytochrome c reductase (NCR) as described (18).

One part of the brain biopsy in twin 2 was also examined biochemically.

Brain biopsy

Twin 2 – Biopsy material from the brain was obtained on two occasions: The first biopsy originated from the right cerebral cortex and subcortical white matter and was removed when multiple subpial transections were made at age 20. Two samples measuring about 4 mm in diameter were used for paraffin embedding and routine histopathology and EM. The second biopsy was taken 7 days later and consisted of the right frontal pole

measuring about 10×7×3 cm in diameter. This material was used for routine histopathology on paraffin material, cryostat sectioning and electron microscopy.

Autopsy

Twin 2 – Autopsy material was fixed in formalin, grossly inspected and selected pieces were embedded in paraffin for staining with HE and the luxol fast blue-cresyl violet method.

Genetics

Southern blot with the multi-locus probe MZ1.3 (Biotest) was used in determining zygosity of the twins. DNA extracted from muscle of both twins and frontal cortex of twin 2 was screened for the A3243G (commonest in MELAS patients) and A8344G (commonest in MERRF) mtDNA mutations. The appropriate amplified polymerase chain reaction (PCR) product was subjected to Apa I digestion to detect the A3243G mutation as reported (5, 6) and Bgl I digestion for the A8344G mutation, using the same primers as reported (19).

Results

The clinical and laboratory findings are summarized in Table 1.

Table 1. Clinical, biochemical and morphological features

		Twin 1	Twin 2
General	Age of onset	12	19
	Height	178 cm	178 cm
	Headache	+	–
	Vomiting	+	–
Brain	Seizures, FSE-EPC, generalised	+	+
	Hemiparesis	+	+
	Hemianopsia	+	?
	Cortical blindness	+	?
	Neuroradiological MELAS	+	+
Mitochondrial angiopathy	?	+	
Muscle	Limb weakness (peripheral)	–	–
	Ptosis	+	+
	RRFs	–	–
	SSVs	–	–
	Biochemical defect	complex I	–
	EM	normal	possibly pathological
Lactate	Plasma	normal	normal
	CSF	normal	normal/high

Seizures and neurological deficits usually occurred related to physical exhaustion, sleep deprivation, or febrile illness in both.

+ = present, – = absent, ? = unknown

Muscle biopsies

In both twins staining for NADH-TR, SDH, COX, and Gomori trichrome were normal. There were no RRFs (Fig. 4), and no increase in fat or glycogen. Blood vessels appeared normal. In twin 1 there were no signs of regeneration or degeneration of muscle fibers. There was some variation in fiber size. In ATPase stain a slight predominance of type 1 fibers was observed. The largest fibers were of type 2. In twin 2 there was mild atrophy of type 2 fibers, chiefly 2B fibers. Muscle fiber distribution and proportions were normal. EM was normal in twin 1. In twin 2 the overwhelming majority of the mitochondria had normal structural appearance. However, some enlarged mitochondria with a watery matrix swelling were present. Some of them were polymorphic with an abnormal position of cristae possibly representing an intravital change (Fig. 5). No paracrystalline inclusions were found.

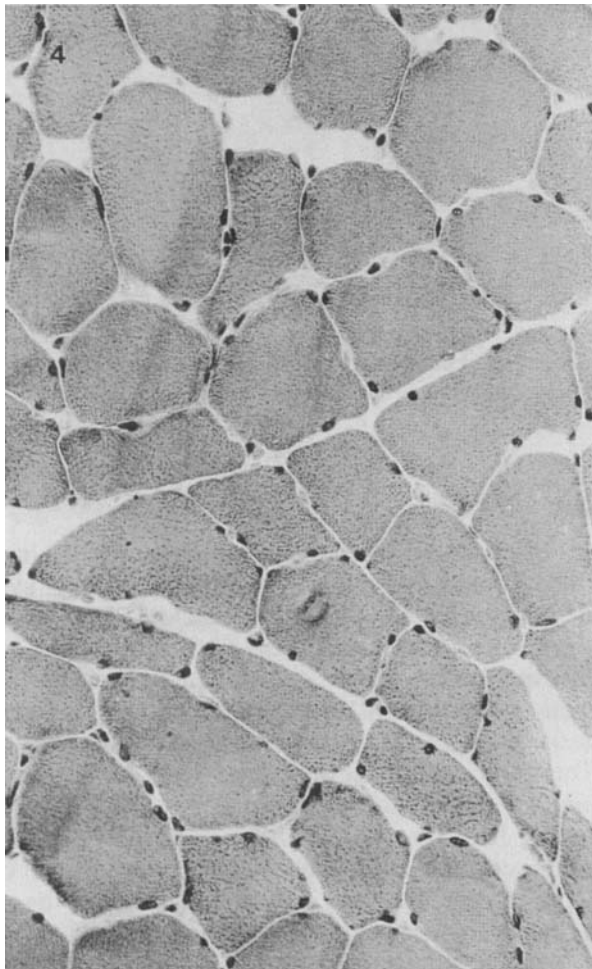


Fig. 4. The biopsy from the vastus lateralis of twin 2 showed a mild atrophy of scattered muscle fibers. No ragged red fibers were present. Gomori trichrome stain.

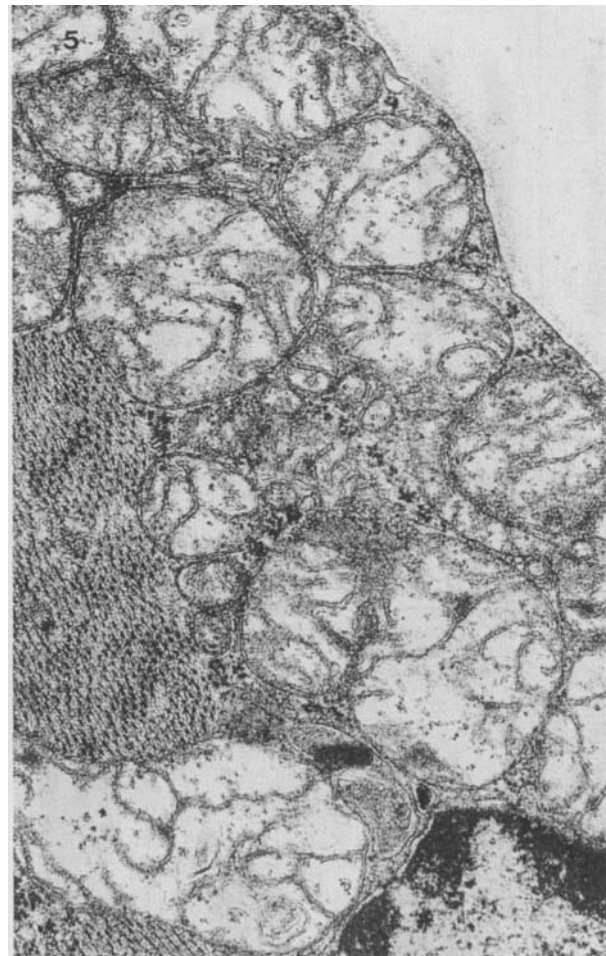


Fig. 5. EM of the muscle biopsy showed that the overwhelming majority of the mitochondria had a normal structural appearance. However, some enlarged mitochondria with a watery matrix swelling were present. Some of them were polymorphic with an abnormal position of the cristae possibly representing an intravital change. No paracrystalline inclusions were found.

Biochemistry

The muscle mitochondrial ATP production rates and enzyme activities in both twins were normal (Fig. 6). However, in twin 1, the low activity of NCR (Fig. 7) in combination with the relatively high ATP production rate with succinate as substrate (Fig. 6) indicates a reduction in complex I activity in the respiratory chain. In Twin 2 no such changes were observed. Biochemistry of the brain tissue did not reveal low levels of any of the complexes in the respiratory chain (compared to that of muscle), but reference values are lacking for the brain.

Brain biopsies – twin 2

The first biopsy investigated by light microscopy showed a few eosinophilic neurons with pyknotic

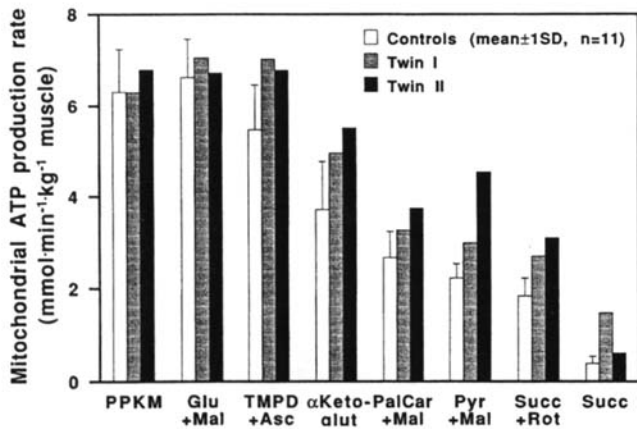


Fig. 6. ATP production in isolated muscle mitochondria in the presence of different substrates in a control group and the twins. For abbreviations see methods.

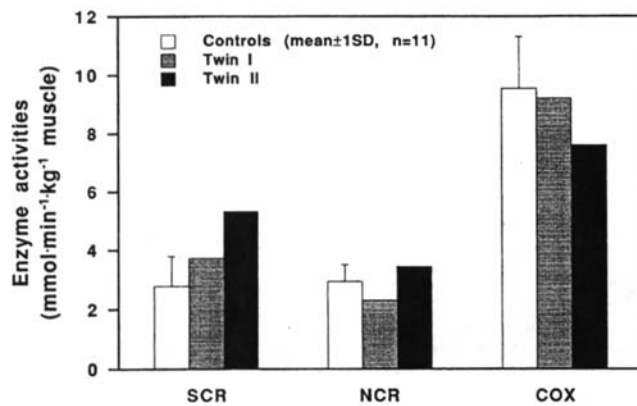


Fig. 7. Respiratory chain enzyme activities in a control group and the twins. For abbreviations see methods.

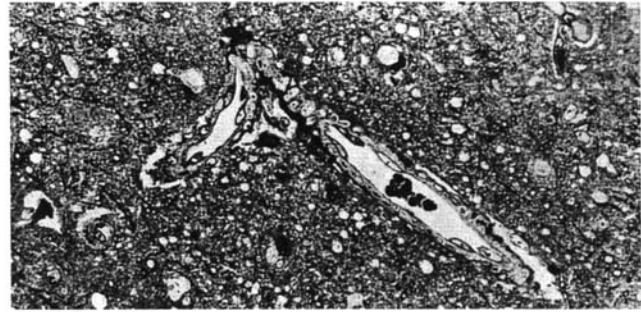


Fig. 8. Brain biopsy twin 2. This epon section showed a cortical microvessel with slight perivascular oedema and large endothelial cell nuclei.

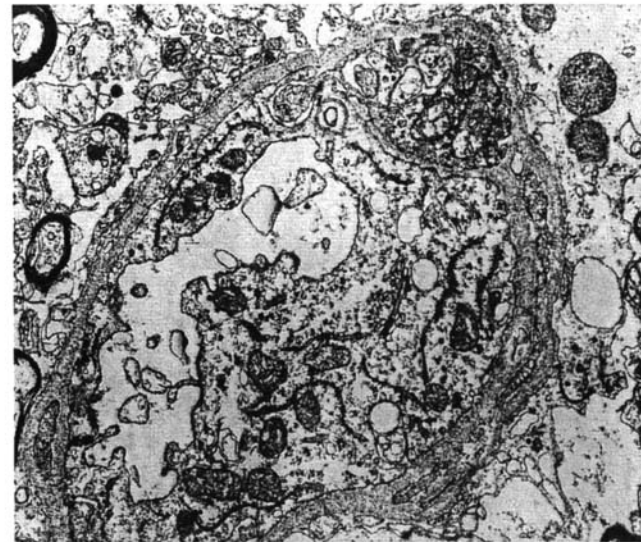


Fig. 9. EM from the same region shown in Fig. 8 showed accumulation of mitochondria both in swollen endothelial cells and pericytes of some intracerebral vessels.

nuclei, i.e. so-called anoxic-ischaemic nerve cell degeneration. The architecture of the cortex was normal and the subcortical white matter showed a mild oedema.

The second biopsy, i.e. material from the resection of the right frontal lobe, showed marked oedema and anoxic-ischaemic nerve cell changes represented by severely shrunken eosinophilic nerve cell bodies. The architecture of the cortex was normal. The cortex and white matter showed some reactive astrocytes. Several intracerebral microvessels had enlarged endothelial cells (Fig. 8). EM showed accumulation of mitochondria in some endothelial cells and pericytes of intracerebral blood vessels (Fig. 9). Some of the mitochondria were polymorphic and had irregular cristae. No paracrystalline inclusions were found. Convincing mitochondrial changes, apart from watery matrix swelling, were not seen in neurons and glial cells.

Autopsy – Twin 2

The brain was markedly swollen with signs of tentorial herniation, flattening of the gyri and compression of the ventricles. The gyral pattern was normal and there was no evidence of malformation or gross focal lesions apart from the resection of the right frontal lobe and the cortical lesions created by the multiple subpial transections.

The cerebral cortex displayed very pronounced changes with extensive laminar necroses, loss of neurons, reactive glial changes particularly astrocytic reactions. There was oedema and pallor of myelin in the cerebral white matter. The basal ganglia, brain stem and cerebellum showed nerve cell loss and gliosis as in the cerebral cortex. The extracranial vessels were normal. Intracerebral vessels had swollen endothelial cells and were often surrounded by oedema.

Genetics

Southern blot with multi-locus probe MZ1.3 revealed that the twins were monozygotic (risk of error < 1/10,000). Neither the A3243G nor the A8344G mtDNA mutations were detected.

Discussion

MELAS is a clinical syndrome with multiorgan involvement characterized by encephalopathy, frequently manifesting as dementia, seizures, or both; stroke before age 40 ys; and evidence of mitochondrial dysfunction in the form of lactic acidosis or RRFs (20). Additional features are normal early development, recurrent headaches, or recurrent vomiting (4).

The disorder in the twins does not fulfill the strict criteria of MELAS, as they lacked RRFs and lactacidaemia. Strongly SDH-reactive blood vessels (SSVs) have been reported in muscle biopsies of MELAS patients (21, 22) and considered diagnostic for mitochondrial involvement. SSVs were not observed in muscle biopsies of the twins. Mitochondrial involvement, however, was present in both. Twin 1 had complex I deficiency in muscle. Twin 2 had mitochondrial angiopathy in brain tissue, similar to findings reported in MELAS (23–25). EM of muscle biopsy in his case revealed abnormal appearance of some mitochondria, possibly representing an intravital change. This twin also had elevated CSF lactate on occasions. Only twin 1 had episodes of headache and vomiting. Early development was not normal, possibly related to eczema, infections, and poor weight gain particularly in twin 1. The A3243G mtDNA mutation, which is the commonest in MELAS patients, was absent in both.

Neuroradiology showed lesions supratentorially, and in twin 2 of the cerebellum in addition. MRI revealed affection of cortical and subcortical white matter. The lesions were fluctuating, compatible with ischaemic lesions in appearance, and led to atrophy. In MELAS predominant areas of lesions are the parieto-occipital or temporal areas (26–29), and multifocal lesions involving cerebral cortex and in some regions the adjacent white matter and the cerebellum have been suggested to be specific for MELAS (28). Not all the lesions in the twins can be explained due to involvement of a major vascular territory, as reported in MELAS (1, 28, 30). Some of the lesions shifted with the passage of time as has been previously reported in MELAS (26–29, 31). Vertebral angiography in twin 1 did not show any stenosis or occlusion, as has previously been reported in MELAS (31–33). Some changes reported in MELAS, such as involve-

ment of the basal ganglia (34) and basal ganglia calcification, were not observed. The changes observed neuroradiologically were judged not to be the result of prolonged seizures. FSE can be accompanied by reversible focal vasogenic cerebral oedema predominantly involving the white matter (35).

The predominant clinical features in both twins were FSE-EPC and generalised seizures, usually occurring in relation to physical exertion, sleep deprivation or febrile episodes. Twin 1 reported a prodrome of hours to days of tiredness and nausea preceding seizures. Neurological deficits, such as hemiparesis in both, and hemianopsia in twin 1, occurred during episodes of seizures. Similar features were reported in an Australian family with mitochondrial disorder where “stroke-like” episodes usually were conveyed by fits (36). Cortical blindness, without preceding seizures, appeared occasionally in twin 1. Severe neurological deficits in twin 1 improved surprisingly well over the years. Clinical muscle involvement apart from ptosis was absent in both. In twin 2 the seizures were intractable and resulted in his death.

Seizures induced by exercise are uncommon in patients with epilepsy (37). The surviving twin is advised to avoid excessive physical exertion. Mobilisation probably should be delayed for several days after seizures disappear, since there is a risk of recurrence. Adequate sleep and rest are advisable. Fever should be investigated and treated.

The clinical implications are that some patients with seizures, stroke-like episodes, and neuroradiological findings suggestive of MELAS have normal lactate levels and lack RRFs and SSVs in muscle, yet suffer from mitochondrial disorder. The signs of mitochondrial involvement were complex I deficiency in muscle in twin 1, cerebral mitochondrial angiopathy and occasionally elevated CSF lactate in twin 2. Some patients with mitochondrial disorder lack evidence of disturbance of energy metabolism. Such an example is a case of Alpers' disease and the A3243G mutation (38). A descriptive eponym for the disorder in the twins at the time of the presentation is mitochondrial epilepsy with stroke-like episodes (MES). However, to our knowledge there are no signs of mitochondrial involvement unique to MELAS. This applies to RRFs, lactacidaemia, specific biochemical defect, mitochondrial angiopathy, or the A3243G mutation, which have been associated with other phenotypes as well. Further molecular analysis of mtDNA may shed light on the pathogenesis of this disorder.

Some differential diagnostic possibilities in patients with seizures in conjunction with stroke-like

episodes are herpes simplex encephalitis (39), focal epilepsy with Todd's paralysis, MELAS, Alpers' disease (40), organic acidurias (4), Rasmussen's encephalitis, acute disseminated encephalomyelitis, vasculitis, and thromboembolic disease. A family history of similar disease may indicate a genetic disorder. Investigations to be chosen from include neuroimaging, EEG, CSF (cells, protein, lactate, virus serology), p-lactate, u-organic acids, muscle biopsy (histochemistry, EM, biochemistry), mtDNA analysis, and screening for autoimmune disorders.

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