

Nonspecific mitochondrial disease with epilepsy in children: diagnostic approaches and epileptic phenotypes

Hoon-Chul Kang · Ji Won Kwon · Young Mock Lee ·
Heung Dong Kim · Hong Jin Lee · Si Houn Hahn

Received: 7 December 2006 / Revised: 23 April 2007 / Published online: 19 June 2007
© Springer-Verlag 2007

Abstract

Objectives This study sought to characterize epileptic phenotypes in children with nonspecific mitochondrial disease (MD) and to evaluate MD diagnostic approaches.

Methods A retrospective analysis of the medical, electroencephalogram, and laboratory records of 142 patients with epilepsy was performed. The patients were evaluated for MD, and 124 patients were included in the final cohort. The MD criteria used included an oral glucose lactate stimulation test (OGLST) and urine organic acid/plasma amino acid (UOA/PAA) assays as metabolic indicators of modified Walker criteria, as suggested by Bernier et al. (Neurology 59:1406–1411, 2002).

This work was supported by the 2004 Inje University Research Grant.

H.-C. Kang · J. W. Kwon
Department of Pediatrics, Epilepsy Center, Sanggye Paik
Hospital, Inje University College of Medicine,
Sang-ye 7 Dong 761-1,
Nowon-gu, Seoul 139-707, South Korea

Y. M. Lee · H. D. Kim (✉)
Department of Pediatrics, Pediatric Epilepsy Clinics, Severance
Children's Hospital, Brain Research Institute, Yonsei University
College of Medicine,
137 Shinchon Dong,
Seodaemun-gu, Seoul 120-752, South Korea
e-mail: hdkimmd@yumc.yonsei.ac.kr

H. J. Lee
Department of Pediatrics, Chuncheon Catholic Hospital, Hallym
University College of Medicine,
Kyo Dong 153,
Chuncheon 200-704, South Korea

S. H. Hahn
Department of Laboratory Medicine and Pathology, Department
of Medical Genetics, Mayo Clinic,
Rochester, MN 55905, USA

Results Twenty-two patients were classified as having definite MD (9), probable MD (5), possible MD (6), or pyruvate dehydrogenase (PDH) deficiency (3), including one patient which showed a respiratory chain (RC) defect and PDH deficiency. Seven out of eight patients in whom significant RC defects were observed showed complex I defects. In 14 patients, epileptic seizures start at infantile ages. Of 17 patients who substantially presented generalized seizures, 4 patients started with partial seizures. Five patients consistently presented only partial seizures. The OGLST and UOA/PAA assays were useful for a more precise diagnosis of MD, although low positive predictive value of the OGLST was regrettable. No patient was classified as definite MD by Walker's original criteria, but the use of our revised MD criteria resulted in the classification of nine additional patients as definite MD.

Conclusions MD manifested considerable diverse epileptic phenotypes and should be considered in the differential diagnosis of epilepsy in children with unexplained encephalomyopathy and progressive and fluctuating clinical courses.

Keywords Mitochondrial disease · Epilepsy · Children · Epileptic phenotypes

Introduction

Mitochondrial diseases (MDs) involve multiple organs and show heterogeneous and unpredictable progression due to the "threshold effect," heteroplasmy, and diverse inheritance patterns [1]. The most common clinical presentation of MD is encephalomyopathy, and epileptic seizures can frequently occur as a presenting sign of mitochondrial encephalopathy [2].

In commonly characterized MD, such as myoclonus epilepsy with ragged red fibers (MERRF) or mitochondrial encephalomyopathies, lactic acidosis, and stroke-like episodes (MELAS), epileptic phenotypes and a definitive molecular diagnosis are well established [3, 4]. However, most nonspecific mitochondrial encephalopathies that present with epilepsy are difficult to diagnose due to the vague diagnostic criteria and enormous variation in clinical progression, especially in children.

The goal of this study is to evaluate a practical, stepwise method for diagnosing MD in childhood epilepsy. Characteristics and relevance of epilepsy in patients who were diagnosed as nonspecific MD were also evaluated by our revised MD criteria, as previously suggested [5, 6].

Materials and methods

The medical, electroencephalogram (EEG), and laboratory records of 142 consecutive patients who visited the Sanggye Paik Hospital from 1997 to 2006 were retrospectively reviewed. The patients presented with repeated seizures of unknown cause. The seizures were accompanied by suspected clinical episodes of MD, and stepwise workups including clinical, metabolic, enzymologic, histologic, and molecular investigations were carried out. Medical records included detailed patient and familial disease history, as well as the results of repeated neuroimaging, echocardiograms, and electrophysiologic studies. Fifty-two patients were followed from their first seizure, and the other 90 patients were referred from other hospitals due to uncontrolled seizures. The mean duration of follow-up was 51.5 months (\pm standard deviation [SD] of 18.6 months). Thirteen patients were excluded from this study due to inadequate medical records, incomplete investigations. In addition, subsequent confirmation of a commonly distinguished MD by means of investigation for mitochondrial DNA (mtDNA) deletions/duplications, and common point mutations (A3243G and T3271C for MELAS, A8344G and T8356C for MERRF), including three patients with MERRF and two patients with MELAS were also excluded.

The initial workups included arterial blood gas, repeated blood and cerebrospinal fluid (CSF) lactate/pyruvate tests (20/124 patients), or oral glucose lactate stimulation tests (OGLST; 104/124 patients). The protocol of OGLST was accorded with that of Chi et al. [7], all patients were required to fast and rest for at least 8 h before the test, baseline levels of blood glucose and lactate were sampled and checked via already indwelled catheter before a 50% glucose/water solution was administered orally at 1.75 g/kg, blood samples then were collected at 30, 60, 90, and 120 min with complete bed rest thereafter. An increase of

>5 mg/dl in blood lactate content at 60 min after glucose loading compared to baseline level was interpreted as significant. Additionally, an organic acid assay with the first urine after non-per os for at least 6 h and an amino acid assay from a blood sample after breakfast were also performed. Urine organic acid (UOA) metabolites were interpreted with MD if lactic aciduria accompanied with an elevation of Krebs cycle metabolites, 3-methylglutaconic acid, ethylmalonic acid, dicarboxylic acids, tiglylglycine, butyrylglycine, 2-ethylhydracrylic, 2-methylsuccinate, and isovalerylglycine was found. Especially, a significant increase in the ratio of lactate to pyruvate and 3-hydroxybutyrate to acetoacetate suggested respiratory chain (RC) defect. In addition, to differentiate with ketolytic defect, which showed a similar pattern of metabolites with MD, an elevation of metabolites even after restriction of protein ($<2 \text{ g kg}^{-1} \text{ day}^{-1}$) for >24 h was also confirmed in MD. In plasma amino acid (PAA) assays, we were looking for secondary hyperalaninemia derived from lactic acidemia and also screened mitochondrial fatty acid disorder by plasma acylcarnitine profiles.

The second step workups were performed on 45 patients who showed either an increased lactate level in repeated blood ($>19.8 \text{ mg/dl}$) and/or CSF ($>22 \text{ mg/dl}$) samplings; an increase in lactate of $>5 \text{ mg/dl}$ 60 min after glucose loading, compared to the initial fasting lactate level in OGLST; or an indication for MD based on UOA or PAA assay results. A muscle biopsy was performed on the vastus lateralis muscle in 32 patients. The tissue was obtained in the operating room and snap frozen in frozen carbon dioxide. Routine morphologic and histochemical staining included periodic acid stain, modified Gomori trichrome stain, ATPase 9.4, nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), and succinate dehydrogenase stain. Electron microscopy was performed in all samples. Spectrophotometric activity assays of the individual RC complexes in cultured skin fibroblasts from 45 patients included NADH-coenzyme Q (CoQ) reductase (complex I), succinate-ubiquinone oxidoreductase (complex II), succinate-cytochrome *c* oxidoreductase (complex II+III), cytochrome *c* oxidase (complex IV), citrate synthase, and pyruvate dehydrogenase (PDH). Unfortunately, functional assays were not routinely performed in our study.

Our revised MD criteria added the OGLST and UOA/PAA assays as part of the metabolic indicators of modified Walker criteria, as suggested by Bernier et al. [6], and the criteria for these indicators were already described (Table 1). The standard of deciding definite (either two major criteria or one major plus two minor criteria), probable (either one major plus one minor criterion or at least three minor criteria), possible (either a single major criterion or two minor criteria, one of which must be clinical), or unlikely RC disorders was utilized as previously suggested [6].

Table 1 Mitochondrial diagnostic criteria applied to 45 children with epilepsies in whom the second step workups for evaluation of mitochondrial disease had been performed

	Major criteria	Minor criteria
Clinical	Clinically complete RC encephalomyopathy ^a or a mitochondrial cytopathy defined as fulfilling all three of the following conditions ^a	Symptoms compatible with an RC defect ^a
Histology	>2% ragged red fibers in skeletal muscle	Any ragged red fibers or >2% SSAM or widespread electron microscopy abnormalities of mitochondria
Enzymology	Cytochrome <i>c</i> oxidase negative fibers or residual activity of a RC complex <30% in a cell line	Antibody-based demonstration of an RC defect or residual activity of an RC complex 30–40% in a cell line
Molecular	Nuclear or mtDNA mutation of undisputed pathogenicity	Nuclear or mtDNA mutation of probable pathogenicity
Metabolic		One or more metabolic indicators of impaired metabolic function including OGLST ^b and urine organic acid ^c /plasma amino acid assays ^d

These criteria are referenced by the suggestions of Walker et al. [5] and Bernier et al. [6]

RC Respiratory chain; RRF ragged red fiber; SSAM subsarcolemmal accumulation of mitochondria; OGLST oral glucose lactate stimulation test

^aThe details of these criteria are described by Bernier et al. [6].

^bAn increase of >5 mg/dl in blood lactate content at 60 min after glucose loading compared to baseline level

^cLactic aciduria accompanied with an elevation of Krebs cycle metabolites, 3-methylglutaconic acid, ethylmalonic acid, dicarboxylic acids, tiglylglycine, butyrylglycine, 2-ethylhydracrylic, 2-methylsuccinate, and isovalerylglycine

^dSecondary hyperalaninemia derived from lactic acidemia

Twenty-two patients, including three patients with PDH deficiency, were assigned a diagnosis of at least possible MD. Epileptic diagnoses of 22 patients were confirmed by single (15 patients) or repeated (7 patients) video EEG monitorings, which were performed according to the International 10–20 system, accompanied by electromyographic recordings from pairs of surface electrodes placed over the shoulder muscles. Epileptic phenotypes and the outcomes of various therapeutic modalities of 22 children were concomitantly evaluated.

Results

Results of stepwise diagnostic investigations

Of 124 patients on whom the first step of the workup was performed, the metabolic results identified 45 patients with possible MD, prompting the second step of the workup. Of the 45 patients who were subjects of the second step, 34 of these patients underwent an OGLST, and all patients underwent an UOA/PAA assay. In 34 patients, the positive and negative predictive value of the OGLST for MD were 30.8 and 75.0% (if the criteria were an increase in lactate of >10 mg/dl, 41.7, and 77.3%), and in addition, the positive and negative predictive value of the UOA/PAA assays were 64.3 and 81.3%, respectively.

Based on the results of the second step, 22 of these 45 patients were classified as having childhood MD with epilepsy: definite MD in 9 patients, probable MD in 5 patients, possible MD in 6 patients, or PDH deficiency in 3 patients. One patient (Patient 3) was classified as both

definite MD, related to a complex I defect and PDH deficiency. Overall, the mean age at presentation of neurologic symptoms of the 22 patients (13 men and 9 women) was 22.7 months (\pm SD of 31.2 months). Among the 22 patients diagnosed by MD with epilepsy, 4 patients (Patients 1, 10, 12, and 13) became subjects of the second-step workups based on the OGLST, without an abnormal increase in lactate level. Three patients (Patients 2, 7, and 8) became subjects of the second-step workups based only on the results of UOA/PAA assays.

All 22 patients naturally displayed clinical manifestations compatible with at least minor clinical criteria: four patients had major clinical criteria, and three patients had a family history that was strongly indicative of a maternally inherited mtDNA mutation. Various multisystem manifestations, such as sensorineural hearing loss, bilateral optic neuropathy, dilated cardiomyopathy, choreic movement, and nutritional problems were observed (Table 2). Except for one patient (Patient 5), who was classified as definite MD related to a complex I defect, and diagnosed by Landau–Kleffner syndrome (LKS), all other patients showed developmental delay and later displayed at least mild mental retardation and/or motor disability.

Of the 32 (out of 45) patients in whom skeletal muscle biopsies were informative, nine patients showed >2% subsarcolemmal accumulation of mitochondria (SSAM) and/or widespread electron microscopic abnormalities of mitochondria including markedly increased mitochondrial size and abnormal shape, an increase in mitochondrial number, abnormal (circular, disoriented, rarified) cristae, osmophilic, or paracrystalline inclusions. One patient showed only significant SSAM and the other one patient

Table 2 Clinical manifestations and epileptic phenotypes in 22 children with mitochondrial disease with epilepsy

No. of patient/sex	First symptom/onset age, months ^a	Final diagnosis	Major/minor criteria (related symptoms)	Seizure types	EEG features	MRI
Pt 1/F	Seizure/6	PDHD	C/M ^b (Dilated cardiomyopathy)	IS→LGS (MC→Abs, GTC)	GSSW, GPFA	Normal
Pt 2/F	Seizure/2	PDHD	C/M ^c (Sensorineural hearing loss)	SMEI (PS→GTC, MC)	GSW	Leuko-malacia
Pt 3/M	Seizure/3	Definite, PDHD	C, E/M (Nutritional)	IS→LGS (Abs, MC)	Hypsarhythmia →GSSW, GPFA	DCA ^d
Pt 4/F	DD/125 (168)	Definite	E/C ^e , M (Nutritional)	PS→MC, AT	FSW→GSW, GPSW ^f	DCA ^d
Pt 5/F	Seizure/54	Definite	E/C, M	LKS	ESES	Normal
Pt 6/F	DD/3(144)	Definite	C/H, M (Nutritional)	GT	GSW ^f	Normal
Pt 7/M	Seizure/64	Definite	C/H, M ^c	Doose syndrome	GSSW, GPSW	Normal
Pt 8/M	Seizure/9	Definite	E/C, H, M ^c	IS→LGS (MC→AT→Abs)	GSSW, GPFA	DCA ^d
Pt 9/M	DD/4 (38)	Definite	C/E, M (nutritional, dysmorphic)	LGS (AT)	Hypsarhythmia →GSSW, GPFA	DCA
Pt 10/M	DD/9 (13)	Definite	E/C, H, M ^b	PS	FSW	Normal
Pt 11/M	Seizure/70	Definite	E/C, H, M, (dysmorphic, bilateral optic neuropathy)	PS→GTC, Abs	FSW→GSW	Normal
Pt 12/M	Seizure/7	Probable	−/C, H, M ^b	SMEI (GTC→MC, PS)	FSW, GSW	Normal
Pt 13/M	DD/24 (51)	Probable	−/C, H, M ^b (chorea)	PS	FSW	DCA
Pt 14/M	Seizure/1	Probable	−/C, H, M	PS	FSW	Normal
Pt 15/F	Seizure/60	Probable	−/C, E, M	PS	FSW	Normal
Pt 16/M	Seizure/17	Probable	−/C, H, M	LGS (AT)	GSSW, GPFA	Normal
Pt 17/M	Seizure/22	Possible	−/C ^e , M	MC, GTC	FSW, GPSW ^f	Normal
Pt 18/M	Seizure/14	Possible	−/C, M	LGS (MC)	FSW →GSSW, GPFA	Normal
Pt 19/M	Seizure/5	Possible	−/C, M	PS	FSW	Normal
Pt 20/F	Seizure/2	Possible	−/C, M	SMEI (PS→GTC→MC)	FSW, GSW	Normal
Pt 21/F	DD/1 (12)	Possible	−/C, M	MC, Abs	GSW, GPSW	Normal
Pt 22/M	Seizure/5	Possible	−/C, M	IS→LGS (AT)	Hypsarhythmia →GSSW, GPFA	Normal

Pt Patient; *C* clinical; *E* enzymologic; *H* histologic; *M* metabolic; *LGS* Lennox–Gastaut syndrome; *MC* myoclonus; *Abs* absence; *GTC* generalized tonic clonic; *SMEI* severe myoclonic epilepsy in infancy; *PS* partial seizure; *AT* atonic; *LKS* Landau–Kleffner syndrome; *GT* generalized tonic; *GSW* generalized sharp and waves; *GSSW* generalized slow sharp and waves; *GPFA* generalized paroxysmal fast activities; *FSW* focal sharp waves; *GPSW* generalized polyspikes waves; *ESES* electrical status epilepticus during sleep; *DCA* diffuse cortical atrophy

^a Seizure onset age in patients who initially presented non-epileptic neurologic symptoms

^b Patients who were indicated by OGLST without an abnormal increase in lactate level

^c Patients in whom the second workups were indicated only by urine organic acid/plasma amino acid assays

^d Patients who showed initially normal neuroimaging

^e Patients with maternal inheritance

^f Patients who showed photoparoxysmal responses

only widespread electron microscopic abnormality. However, none of these samples showed any ragged red fibers.

In six patients, the activity of complex I levels in cultured fibroblasts was reduced to 20–30% of normal. Additionally, one patient showed 30–40% of the normal activity of complex I, and one patient showed 30–40% of the normal activity of complex IV. However, all patients showed at least more than 20% activity level for all RC complexes. Three of nine patients who were classified as definite MD and five of six patients with probable MD did

not display any significant RC defects, in spite of the wide enzymologic criteria.

Four of nine patients who were classified as definite MD and one of five with probable MD showed diffuse cortical atrophy without focal lesions. The initial magnetic resonance imaging (MRI) findings were nonspecific in three patients (Patients 3, 4, and 8) in whom the initial MRI was potentially informative. In addition, one patient (Patient 2) who was confirmed by PDH deficiency showed an encephalomalatic change in the right temporal region

without any history of perinatal insult; this change possibly occurred in the antenatal period.

The use of modified Walker's criteria [6], when compared to Walker's original criteria [5], resulted in the classification of eight additional patients as definite MD. One additional patient was classified as definite MD by our revised MD criteria including an OGLST and UOA/PAA assays as metabolic indicators. No patient presented symptoms that met Walker's original major diagnostic criteria [5] (Fig. 1).

Epileptic phenotypes of 22 patients with MD

Epileptic diagnoses of 22 patients included Lennox–Gastaut syndrome (LGS) in 7 patients, severe myoclonic epilepsy in infancy (SMEI) in 3 patients, Doose syndrome in 1 patient, LKS in 1 patient, nonspecific generalized seizure disorders, including myoclonic jerkings, atypical absence, and atonic seizure, in 5 patients, and consistent partial seizure disorders in 5 patients. Initially, non-epileptic neurological symptoms were observed in six patients who showed developmental delay before the onset of epileptic seizures. However, seizures were the first unequivocal neurologic sign in 16 patients. In 14 patients, epileptic seizures start at an infantile age (mean±SD, 8.5±6.3 months); however, in two patients (Patients 4 and 6), seizures onset when the patients were teenagers. Out of ten patients who had myoclonic seizures, two patients' (Patient 4, definite MD, and Patient 17, possible MD) seizures were accentuated by photic stimulation. There was one additional patient (Patient 6) who also showed photoparoxysmal responses. Of 17 patients who presented generalized seizures, four patients started with focal and/or secondary generalization, which were identified with routine and video EEG monitoring, subsequently followed by generalized seizures such as myoclonus, atypical absence, or atonic seizures after a certain period (mean±SD, 39.3±15.9 months). Five

patients consistently presented only partial and/or secondary generalization seizures during follow-up (mean±SD, 89.4±47.3 months) without any generalized epileptic phenotypes. All patients were prescribed a supplementary mitochondrial cocktail therapy including CoQ 10 at a dose of 5 mg/kg per day, L-carnitine at a dose of 66 mg/kg per day, riboflavin at a dose of 5 mg/kg per day, and multivitamins. The mitochondrial therapy overlapped with other therapeutic modalities; thus, specific results could not be analyzed. However, the parents of eight patients reported an improvement of cognition, motor activity, gait disturbance, and general well-being while their children were undergoing mitochondrial cocktail therapy.

The detailed epileptic phenotypes of the 22 MD patients are summarized in Table 1, and several characteristic patients are described below. In all three patients (Patients 1, 2, and 3) with PDH deficiency, the first symptoms were prolonged intractable seizures including LGS (Patients 1 and 3) and SMEI (Patient 2), with infantile onset followed by developmental deterioration. All patients showed the typical EEG features of their respective epileptic syndromes, but accentuation by photic stimulation was not observed. The ketogenic diet (KD) had been tried in all patients with PDH deficiency, but did not show the expected favorable progress. Patient 1 died during the course of the KD. This patient showed dilated cardiomyopathy despite initially normal cardiac function and periodic echocardiography.

Patient 4, who was classified as definite MD, was well until age 12, except for her learning disability. At age 12, she started having frequent partial seizures with secondary generalization, and at age 14, she showed typical myoclonic jerkings with generalized polyspike wave discharges (photoparoxysmal response). Her symptoms were accentuated by photic stimulation, and she showed subsequent general weakness. Five months after the onset of frequent myoclonic jerkings, atonic seizures also appeared. Patient 4 had a family history of a similar clinical progression in her mother and her already deceased grandmother and uncle. A complex I defect was confirmed; however, no mtDNA point mutations (A8344G and T8356C for MERRF) or ragged red fibers were observed.

Patient 5 was diagnosed with LKS and definite MD related to a complex I defect. Her cognitive deterioration and electrical status epilepticus during sleep could not be controlled by other therapeutic modalities, including newly developed antiepileptic drugs, prednisone, or intravenous immunoglobulin. The KD was cautiously added with supplementary mitochondrial cocktail therapy. The patient became seizure-free and showed normal EEG findings within 1 month of beginning the diet. After successful completion of the diet for 2 years, she maintained normal neuro-cognitive activities with continuing mitochondrial cocktail therapy

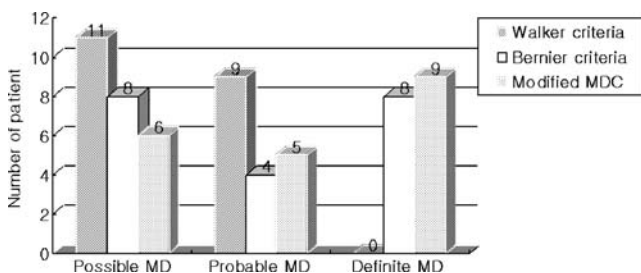


Fig. 1 Comparison of various mitochondrial diagnostic criteria (MDC) including that of Walker et al. [5] and Bernier et al. [6] and our modified criteria including an OGLST and UOA/PAA acid assays as metabolic indicators in 20 patients who were diagnosed by MD related to RC complex deficiency. MD Mitochondrial disease. The criteria of Bernier et al. [6], along with our subtly revised criteria could diagnose more patients with MD as compared to Walker's criteria [5] for adult MD

only. After a trial of discontinuation of the mitochondrial cocktail therapy, she complained generalized weakness and has since been maintained on cocktail therapy. This patient had been already reported detailedly in another article [8].

Patient 6 had no specific perinatal problems, but did show a developmental delay and severe failure to thrive since birth. She started having frequent generalized tonic seizures with photoparoxysmal responses at age 12. She had no familial history of any similar clinical symptoms, and an RC defect was not confirmed. However, she was classified as definite MD with typical clinical manifestations and electron microscopic findings of the skeletal muscle biopsy. Additionally, her seizures and general well-being were improved after supplementary mitochondrial cocktail therapy.

Patient 10 was classified as having probable MD due to a complex I defect and the electron microscopic findings of the skeletal muscle biopsy and was moved to the second step of the investigation due to an increased level of lactate (>5 mg/dl) in the OGLST without an overall abnormal increase in lactate level. His first symptom was developmental delay, identified immediately before his first birthday, and later, he presented with consistent focal seizures. Soon after he was diagnosed, his younger brother showed exactly the same clinical progress, but his parents refused further diagnostic studies.

Discussion

It has been clinically and experimentally recognized that MD can cause epileptic seizures [9]. However, the results of previous investigations into diagnosing MD in epileptic children have been intermediate or ambiguous. Fortunately, modified mitochondrial diagnostic criteria for children have been proposed and provide the clinician with a tool to weigh and integrate the clinical findings and laboratory information from a number of sources for diagnosing MD [6]. However, these extensive workups are often restrictive due to the technical difficulties in assaying RC complexes and the difficult interpretation and invasiveness of muscle biopsy. To increase the positive and negative predictive value of metabolic studies for screening MDs, additional criteria such as exercise ergometry or the OGLST were suggested [7, 10]. In this cohort, the OGLST was used, and in four patients where the OGLST was significant without an abnormal increase in lactate. We also utilized UOA/PAA assays as metabolic indicators of minor criteria, and in three patients, the UOA/PAA assays were the only predictors of MD. The OGLST and UOA/PAA assays were often useful for a more precise prediction of MD; however, the low positive predictive values were discouraged, especially for OGLST.

The criteria of enzyme activity is somewhat arbitrary because activity levels are continuous data, without any

obvious boundary between normal and abnormal values. In addition, heteroplasmy in cells and tissues can cause the lower limits for normal skeletal muscle RC complexes to vary widely between individual centers [11]. We utilized Bernier's suggestion [6] of more generous criteria and, additionally, added OGLST and UOA/PAA assays as metabolic indicators, and nine patients displayed significant RC defects (seven patients with definite MD and one patient with probable MD). In an additional seven patients with one patient of probable and six patients of possible MD, enzyme defects were strongly suggested, but the patients were not in the range of the MD criteria as defined by Bernier et al. [6]. Fourteen patients, almost two thirds of the patients with MD, could be classified as having MD based on typical clinical features and the findings of the skeletal muscle biopsy. It is recognized that as fibroblasts may not exhibit an RC defect despite an abnormal muscle RC result, and have high rotenone insensitive complex I activity, there is a significant chance that RC defects may be missed in fibroblasts. However, our data suggest that even a more extensive enzymologic range of criteria may be required for proper diagnosis.

Ragged red fibers are more characteristic in MD than the SSAM and mitochondrial abnormalities in electron microscopy, but because typical ragged red fibers normally appear with aging, SSAM and widespread electron microscopic abnormalities are sometimes more informative when diagnosing MD in children [12]. We also did not observe any ragged red fibers in 32 skeletal muscle biopsies.

Non-epileptic neurological symptoms were initially seen in six patients who showed delayed development combined with various MD-related multisystem manifestations before the onset of epileptic seizures. However, seizures were the first unequivocal neurologic sign in almost two thirds of the cases, especially in infantile epilepsies. As previously reported by other authors [13], unfortunately, there were no consistent epileptic phenotypes related to nonspecific MD. Most infants consistently presented partial seizures, although subsequent generalized seizures often followed with aging. A part of typical generalized epilepsies occurred in infancy were presented with infantile spasms.

In our patients, the RC defects were seen mostly in complex I, with seven patients showing this defect, and less often in complex IV, with one patient showing a complex IV defect. Complex I deficiencies are the most common cause of MD, especially in infants with epilepsy [12]. Although our data does not speak to epidemiologic trends due to the selection bias, it is consistent with complex I deficiency being the most prevalent RC defect in childhood epilepsies.

PDH deficiency is fairly common in MD related to childhood epilepsy and can present diverse clinical progressions depending on the specific defects and genetic background of the patient [13]. Wada et al. [14] reported

PDH deficiency that presented with infantile spasms and antenatal origin of brain damage. We also observed a similar clinical presentation and neuroimaging findings in Patient 2, although epileptic phenotype was SMEI, and the antenatal imaging was not useful. Similar to other observations [13] that repeated seizures sometimes precede brain damage in MD, our three patients showed initial normal findings, followed by progressive cortical atrophy.

EEG features varied according to seizure types, and contrary to the commonly distinguished MERRF or MERRF-like syndrome [3, 4], nonspecific MD with epilepsy did not often present with typical photoparoxysmal responses. The EEG findings were more consistent with their respective epileptic syndromes or seizure types rather than with a diagnosis of MD.

Most patients were resistant to the effects of antiepileptic drugs. A high-fat diet (the KD) has been recommended as an alternative source of acetylcoenzyme A in patients with PDH deficiency [15]. However, overall, this diet failed to help patients in our study. Several conditions, such as fatty acid oxidation defect and pyruvate carboxylase deficiency, would be adversely affected by the KD [16]. There are still controversies concerning the efficiency of KD in mitochondrial RC defect [16, 17]. Santra et al. [17] illustrated that the demonstration that ketone bodies can distinguish between normal and respiratorily compromised cells in vitro points to the potential use of a KD to treat patients with heteroplasmic mtDNA disorders. We also experienced clinically that the KD was tolerated with supplementary mitochondrial cocktail therapy in one patient with LKS related to a complex I defect [9]. In addition, we observed improvement of seizure outcomes and improvement in neurologic disabilities after supplementary mitochondrial cocktail therapy in some patients, although the analysis was performed at a qualitative level. Fernando et al. [18] reported that patients with cardiomyopathy had a higher mortality rate than those without cardiomyopathy. During the course of our study, one patient died from respiratory distress syndrome accompanied by dilated cardiomyopathy during the KD. Although this patient's energy failure was possibly due to prolonged poor dietary intake, resulting from refusal of the fat diet, it may be presumed that MD patients with cardiac problems have a higher risk of complications.

In conclusion, the discovery of mitochondrial encephalopathy in children with epilepsy requires various diagnostic tools. The OGLST and the UOA and PAA assays were often useful for screening the MD in children, although low positive predictive value of the OGLST was regrettable. Although our retrospective data cannot provide definite epidemiologic information concerning the frequency of epilepsy in MD, MD is more common in epilepsy patients than previously thought [19], and the presence of heterogeneous and nonspecific epileptic phenotypes with diverse

clinical features in our patients suggest that MD should be considered in the differential diagnosis of epilepsy in children with unexplained encephalomyopathy and progressive and fluctuating clinical courses.

References

1. Vu TH, Hirano M, Dimauro S (2002) Mitochondrial diseases. *Neurol Clin* 20:809–839
2. Schmiedel J, Jackson S, Schäfer J, Reichmann H (2003) Mitochondrial cytopathies. *J Neurol* 250:267–277
3. Berkovic F, Carpenter S, Evans A, et al. (1989) Myoclonus epilepsy and ragged-red fibers (MERRF). A clinical, pathological, biochemical, magnetic resonance spectrographic and positron emission tomographic study. *Brain* 112:1231–1260
4. Pavlakis SG, Philips PC, Dimauro S, et al. (1984) Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes: a distinctive clinical syndrome. *Ann Neurol* 16:481–488
5. Walker UA, Collins S, Byrne E (1996) Respiratory chain encephalomyopathies: A diagnostic classification. *Eur Neurol* 36:260–267
6. Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR, (2002) Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* 59:1406–1411
7. Chi CS, Mak SC, Shian WJ, Chen CH (1992) Oral glucose lactate stimulation test in mitochondrial diseases. *Pediatr Neurol* 8:445–449
8. Kang HC, Kim HD, Lee YM, Han SH (2006) Landau-Kleffner syndrome with mitochondrial respiratory chain-complex I deficiency. *Pediatr Neurol* 35:158–161
9. Kunz WS (2002) The role of mitochondria in epileptogenesis. *Curr Opin Neurol* 15:179–184
10. Haller RG, Lewis SF, Estabrook RW, DiMauro S, Servidei S, Foster DW (1989) Exercise intolerance, lactic acidosis and abnormal cardiopulmonary regulation in exercise associated with adult skeletal muscle cytochrome c oxidase deficiency. *J Clin Invest* 84:155–161
11. Wolf NI, Smeitink JAM (2002) Mitochondrial disorders. *Neurology* 59:1402–1405
12. Kirby DM, Crawford M, Cleary MA, Dahl HM, Dennett X, Thorburn DR (1999) Respiratory chain complex I deficiency. *Neurology* 52:1255–1264
13. Canafoglia L, Franceschetti S, Antozzi C, et al. (2001) Epileptic phenotypes associated with mitochondrial disorders. *Neurology* 56:1340–1346
14. Wada N, Matsuishi T, Nonaka M, Naito E, Yoshino M (2004) Pyruvate dehydrogenase E1 α subunit deficiency in a female patient: evidence of antenatal origin of brain damage and possible etiology of infantile spasms. *Brain Develop* 26:57–60
15. Wexler ID, Hemalatha SG, McConnell J, et al. (1997) Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets. *Neurology* 49:1655–1661
16. Nordli DR Jr, DeVivo DC (2001) The ketogenic diet. In: Wyllie E (ed). *The treatment of epilepsy, principles & practice*, 3rd edn. Lippincott Williams & Wilkins Co., Philadelphia, pp 1001–1006
17. Santra S, Gilkerson RW, Davidson M, Schon EA (2004) Mitochondrial DNAs in cultured human cells. *Ann Neurol* 56:662–669
18. Scaglia F, Towbin JA, Craigen WJ et al. (2004) Clinical spectrum, morbidity, and mortality in 113 pediatric patients with mitochondrial disease. *Pediatr* 114:925–931
19. Munnich A, Rötig A, Cormier-Daire V, Rustin P (2001) Clinical presentation of respiratory chain deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The metabolic & molecular bases of inherited disease*, 8th edn. Lippincott McGraw-Hill Co., New York, pp 2261–2274