Multimodal Evoked Potentials in Three Siblings with Mitochondrial Disease

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Abstract- Mitochondrial diseases are heterogeneous disorders affecting multiple systems. Here, we presented the findings of multimodal evoked potential (EP) studies of three siblings with a specific A8344G mutation of mitochondrial DNA. One of them had DM and another two had a history of encephalopathy. Visual EPs were abnormal in one patient and motor, somatosensory and brainstem auditory EPs were observed in all three patients. Our EP studies showed that the A8344G mutation of mitochondrial DNA involved multiple levels of the central nervous system even though there were no correlated symptoms. Therefore EP is an adjunct of methods to detect the functional disturbance and to screen the distribution of the involvement of the nervous system in mitochondrial diseases.

Key Words: A8344G, DNA mutation, Evoked potentials, Mitochondria

Acta Neurol Taiwan 2006;15:92-97

INTRODUCTION

Mitochondrial diseases are a group of disorders due to either sporadic or inherited mutations in nuclear and or mitochondrial DNA-located genes[(1)]. These heterogeneous disorders usually affect multiple systems and present a diagnostic challenge because of their wide variations in presentation and course. Several point mutations in mitochondrial DNA have been recognized to be causative for specific phenotypes and syndromes of mitochondrial diseases. However, the genotype-phenotype correlation is not fully established. The mutation of mitochondrial DNA A8344G results in a mutation of mitochondrial tRNA for lysine and is one of the most extensively studied mutations[(1)].

A8344G patients demonstrate highly age-related and tissue-related clinical variability, contributed to the characteristics of mitochondrial genetics, such as mitotic segregation, heteroplasmy, and the threshold effect[(1)]. Therefore, the manifestations of mitochondrial DNA A8344G have varied a lot including: myopathy[(2-8)], chronic progressive external ophthalmoplegia[(9)], mental impairment[(3,7,10)], encephalopathy[(5,6,10)], myoclonus[(2-4,6-8)], intention tremor[(7)], ataxia[(2-4,6,8)], hearing loss[(2,3,6-8)], retinopathy[(4)], optic neuropathy[(7)], peripheral neuropathy[(4,6)], seizures[(3,4,8)], lipomas[(4,10)], small stature[(4)],

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Received November 24, 2005. Revised January 9, 2006.
Accepted March 10, 2006.

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fatigue[3,4], and stroke-like episode[11]. Basically, a higher percentage of mutated mitochondrial DNA is usually associated with a more serious form of the disease, but there is not always a correlation between the degree of heteroplasmy and the severity of the disease or the age of the first clinical symptom[1]. Though mitochondrial DNA A8344G is often observed in patients with myoclonic epilepsy and ragged red fibers (MERRF), not all A8344G patients present with MERRF[9,11], indicating that the A8344G mitochondrial DNA mutation does not always involve specific neurons or glial cells of the central nervous system (CNS).

Multimodal evoked potentials (EPs) can help evaluate indirectly the amount of demyelination and axonal loss in the central nervous system of patients by exploring the functional consequences of the disease process. Multimodal EPs also provide information about CNS structures, such as the spinal cord and the optic nerves which are not as well visualized by MRI. As it was hard to quantify the proportion of mutant mitochondrial DNA in the CNS, we used multimodal EPs to assess the function of the central sensory and motor pathways of patients with an A8344G mutation of mitochondrial DNA, and also to see if a specific phenotype of A8344G mitochondrial disease had specific EP patterns.

MATERIALS AND METHODS

Subjects

We included three patients with mitochondrial DNA A8344G from the same family. The demographics and clinical findings are listed in Table 1. They all fit the diagnostic criteria of mitochondrial disease and were diagnosed by Southern blot analysis[10]. All patients were treated with thiamine and co-enzyme Q now. Their ages at diagnosis were 33, 31 and 25 respectively. All the three patients showed higher serum lactate levels (27.4 mg/dl, 20.3 mg/dl, 30.7 mg/dl; control 5-15 mg/dl) respectively. Ophthalmoplegia was noted in case 3 at the stage of subacute encephalopathy. Their data were compared with age- and sex-matched control subjects after informed consent.

Table 1. *Demography of patients with an A8344G mutation*

Patients	1	2	3
Age (yr)	33	31	25
Sex	male	male	male
Height (cm)	163	170	156
MMSE	26	30	23
IQ	ND	ND	81
Encephalopathy	+	-	+
Seizure	+	-	-
Fatigue	+	-	+
Lipomatosis	-	-	+
Diabetes	-	+	-
Ophthalmoplegia	-	-	+
MRI findings	-	-	+
Neuropathy	-	+	+
Myopathy	+	+	+
EEG	+	-	+

yr: year; +: present or abnormal; -: absent or no significant findings; ND: not done. MRI: magnetic resonance images; EEG: electroencephalography.

EP studies

Visual EPs (VEPs) were obtained by simulating at a 3-degree angle to specifically assess demyelination of maculofoveal pathways. The criteria for VEP abnormalities were an absence of P100, a prolonged P100 latency, or a side difference of P100 amplitude and latency beyond 2.5 standard deviation (SD) of the mean.

Brainstem auditory EPs (BAEPs) in response to 1024 10 Hz click stimuli were measured bilaterally, focusing on the interpeak latencies of I-V, I-III and III-V. The following patterns of BAEPs were considered abnormal: prolonged peak latency and an increased inter-peak interval beyond 2.5 SD of the mean. While the V/I amplitude ratio was greater than 300%, wave I was considered to be too small and was taken as an index of peripheral hearing impairment. When the V/I amplitude ratio was less than 0.5, central impairment was considered.

Somatosensory EPs (SEPs) were obtained from the upper and lower limbs by electrical stimulation delivered to the median nerve at the wrist and the posterior tibial nerve at the ankle. Responses were recorded from the ipsilateral Erb's point (N9), above the 5th cervical spine (N13) and the contralateral scalp (N20) with reference to

Table 2. *Evoked potentials of patients with an A8344G mutation*

Patient	1		2		3		Control
	Left	Right	Left	Right	Left	Right	
VEP							
P100 (ms)	91.2	93.9	141.0*	137.4*	107.5	109.6	98.4 ±4.9
BAEP							
I (ms)	1.48	DR	1.60	DR	DR	2.24*	1.68 ±0.14
III	4.14	DR	4.18	DR	DR	DR	3.73 ±0.18
V	5.96	DR	6.00	DR	DR	6.02	5.48 ±0.23
I-III	2.66*	DR	2.58*	DR	DR	DR	2.01 ±0.18
III-V	1.82	DR	1.82	DR	DR	DR	1.97 ±0.16
Median N SEP							
N9 (ms)	10.6*	11.1*	DR	DR	9.60*	8.95	8.8 ± 0.6
N13	DR	DR	DR	DR	DR	DR	12.6 ± 0.6
N19	21.50*	22.40*	25.00*	25.75*	20.00	18.85	19.2 ± 0.7
N9-N19	10.9*	11.3*	DR	DR	10.4	9.9	9.3 ± 0.5
Tibial N SEP							
N22 (ms)	21.8	22.7	DR	DR	DR	DR	21.5 ±1.2
P37	42.2	42.1	DR	52.1*	DR	DR	37.6 ±2.2
Amplitude (μV)							
P37-N45	0.21*	0.32*	DR	0.13*	DR	DR	1.9 ±0.4
MEP of hand muscles to TMS							
MT (%)	90*	85*	65*	70*	70*	70*	45.2 ± 7.0
Latency	22.1*	23.8*	30.6*	29.6*	23.2*	22.8*	19.2 ± 0.8
CMCT	7.13	7.82*	9.23*	9.12*	8.28*	7.92*	6.4 ± 0.5

*: abnormal with data beyond the mean with more than 2.5 SD; DR: difficulty in recognition or measurement; VEP: pattern-reversal visual evoked potentials; BAEP: brainstem auditory evoked potentials; SEP: somatosensory evoked potentials; MEP: motor evoked potentials with transcranial magnetic stimulation; MT: motor threshold; hand muscle: abductor pollicis brevis; CMCT: central motor conduction time.

Table 3. *A comparison of evoked potential studies of mtDNA A8344G patients*

	Case/32	VEP/9	BAEP/11	SEP/27	MEP/12
Lai 2006	3	A1	A3	A3	A3
Tsutsumi 2001	3	ND	A3	ND	ND
Di Lazzaro 1997	6	ND	ND	A6	A0
Arpa 1997	5	A1	A2	A3	A0*
Thompsson 1994	5	ND	ND	A5	ND
Piccolo 1993	7	D	ND	A3	ND
Smith 1993	1	A0	ND	A0	ND
Ohtsuka 1993	2	A2	A2	A0	ND
Total	A26	A4	A10	A20	A3

A: number of patients with abnormal findings in the previous reports; ND: not done; *: TMS was studied in 3 of 5 patients in the study.

Fz for median nerve stimulation; and the 1st lumbar spine -contralateral iliac crest (N22), the 5th cervical spine (P30), and the contralateral scalp (P39) for posterior tibial nerve stimulation. Five hundred responses were averaged for each trial of median nerve stimulation and 1000 responses for tibial nerve stimulation. Results for SEPs were considered abnormal at the following conditions: the peak amplitude of N20-P25 was less than 0.9 μV; and the central conduction time (CCT), or peak latencies were beyond 2.5 SD of the mean.

Transcranial magnetic stimulation (TMS) with recording on the abductor pollicis brevis of the upper limb was performed with a magnetic stimulator (MagStim 200, Magstim Company, UK) to measure the motor evoked potential (MEP) latency. The motor CCT

Table 4. *The correlation between brain imaging studies and evoked potentials*

	Piccolo (7)		Lai (3)		Smith (1)		Arpa (2)		Ohtsuka (2)		di Lazzaro (6)		Total	
Imaging\EP	N	A	N	A	N	A	N	A	N	A	N	A	N	A
N	4	2	0	2	1	0	0	1	0	0	0	3	5	8
A	0	1	0	1	0	0	0	1	0	2	0	3	0	8

( ): the total number of patients who had brain imaging studies in the report; N: normal; A: abnormal

was calculated by subtracting the peripheral motor conducting time from the total latency of the MEP, i.e. motor CCT (ms) = cortical MEP latency - (F wave latency + distal CMAP latency - 1)/2. Results for MEPs were considered as abnormal if the cortical evoked potentials were absent, or if the motor CCT or the side-to-side differences in motor CCT were beyond 2.5 SD of the mean.

We made a comparison with other EP studies of the A8344G mutation of mitochondrial diseases. Few published EP studies specific to A8344G mitochondrial disease could be found in a Medline search. We were able to collect another 29 patients from the literatures, for a total 32 patients, in the Table 3. In these 32 patients, VEPs were done in 9 patients, BAEPs in 11, SEPs in 27, and MEPs in 12. All of these patients had been proven to have mtDNA A8344G mutation and had had at least one EP study[2-8]. Brain imaging studies were done in 21 of 32 patients (Table 4).

RESULTS

Electroencephalography showed diffuse slow background activities without epileptic discharges or focal abnormalities in patients 1 and 3. Mild peripheral neuropathy with mild conduction slowing was diagnosed in patients 2 and 3. The results of multimodal EPs were listed in Table 2.

Delayed VEP latencies were noted in patient 2 only. Inconsistent BAEPs were noted in our patients, especially the wave III. A prolonged interval of I-III was recognized in patients 1 and 2. Abnormal SEPs were noted in our 3 patients. Median nerve SEPs showed decreased amplitudes of N9 and N13 in the 3 patients, delayed latencies of N9 in patients 1 and 3, delayed latencies of N19 in patients 1 and 2, and prolonged N9-N19 intervals in patient 1. In the tibial nerve SEPs, the chief findings were decreased amplitudes of the cortical potentials in our 3 patients. TMS studies showed a prolonged central motor conduction time and decreased cortical excitabilities in our patients.

In summary, 26 of 32 patients (81.3%) with A8344G mitochondrial DNA mutation disease had at least one EP abnormalities (Table 3). Though most brain imaging findings were not consistent with EP findings, we took abnormal brain imaging as another index of neurological dysfunction. Abnormal brain imaging was noted in 8 but abnormal EPs in 16 of 21 patients. Five patients had normal EP studies and normal brain imaging. The agreement between the brain imaging and EP results was poor (kappa value = 0.32) (Table 4).

DISCUSSION

Pattern-reversal VEPs are a method to evaluate optic nerve function. A delayed VEP latency was noted in patient 2 similar to the other reports[4,7]. In the report of Arpa et al.[4], VEPs showed an absent response in one and delayed P100 latencies in one[4]. None of the patients had retinal degeneration. In the series of Ohtsuka et al.[7], P100 latencies of pattern-reversal VEPs were prolonged throughout the clinical course, even with the intervention of medical management. Abnormal VEPs were consistent with the clinical observation of Ohtsuka et al.[7], that optic nerve could be involved in A8334G mitochondrial diseases.

BAEPs are a simple method to screen hearing function and its related brainstem function. Abnormal BAEPs were noted in our patients despite the fact that none of them complained of hearing loss. In the report of Tsutsumi et al.[8], abnormal BAEPs were also noted in their 3 patients; 2 with increased inter-peak latency of I-V and 1 with delayed wave I latency. All their manifes-

tations were mitochondrial encephalopathy, ragged red fibers and hearing loss[8]. Taking these results together with those of audiometry and electrocochleograpy, they concluded that the primary lesion underlying hearing loss was in the cochlea. In the series of Ohtsuka et al.[7], 2 siblings had hearing impairment and also had abnormal BAEPs with prolonged interpeak latency of III-V. In the series of Arpa et al.[4], decreased BAEP amplitudes were noted in 2 patients with ataxia[4].

SEPs are well known as a method to assess the function of the dorsal column and its related supraspinal pathway. Abnormal SEPs were reported not only in our patients but also in the other series[3-6]. Giant SEPs indicate cortical hyperexcitability and were noted in A8344G patients with cortical myoclonus[3,5]. Though MERRF is often observed in A8344G patients, none of our patients had myoclonic epilepsy. In A8344G patients without myoclonus or with myoclonus not of cortical origin, SEPs showed decreased peak amplitudes or delayed latencies of cortical responses in the series of Arpa et al.[4], and even normal responses in the case of Smith et al.[2]. These EP studies showed that there was no specific pattern in A8344G patients.

Pathological studies have proved that the spinal cord and its corticospinal tracts are involved in MERRF[12]. Our TMS results confirmed their findings and indicated that mitochondrial DNA A8344G disease may also involve the corticospinal tract as other phenotypes of mitochondrial diseases. Abnormal MEPs have been reported in mitochondrial patients with Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia, encephalopathy with or without myopathy, encephalopathy with lactic acidosis and stroke-like episodes, myo-neuro-gastrointestinal-encephalopathy, and pure myopathy[5].

The literature review as well as our study results showed that A8344G mitochondrial disease is characterized by a wide range of clinical features, with involvement of multiple levels of the nervous system. The overall incidence of EP abnormalities was 81.3% in patients with A8344G mitochondrial disease, even in a patient with pure myopathy and in an asymptomatic subject (case II-2 of Piccolo et al.[6]; case 13 of di Lazzaro et al.[5]). EP abnormalities did not have a significant correlation with the clinical features of mitochondrial disease. Our study showed that a specific phenotype of A8344G mitochondrial disease did not produce specific EP patterns.

The discrepancy between EPs and clinical manifestations has been reported in many neurological diseases, such as in multiple sclerosis[13]. In sensory symptomatic subjects with normal EP studies, it is argued that routine electrophysiological studies may not reflect the functional deficits due to small fibers, neuronal loss, or axonal lesions. In sensory asymptomatic subjects with abnormal EP studies, it is possible due to the following mechanisms; 1) The process of chronic or congenital diseases is so slow that patients may adapt themselves without significant complaints; 2) Neuroplasticity compensates the physiological function and makes the clinical manifestations as minimal as not to be detected; 3) Mitochondria disease may involve multiple organs and tissues concomitantly but with different degrees of severity. The major problems may mask the minor deficits so that patients and clinicians may neglect without attention; for example, patients with cognitive disorders due to mitochondrial encephalopathy may not well express themselves in details.

EPs have been widely used to study the function of the central motor and sensory pathways in humans. The responses it evokes are thought to be mediated by fast conducting pathways. However, mitochondrial dysfunction chiefly involves neurons and then myelin. The major neuropathology of mitochondrial diseases consists of spongy degeneration, neuronal loss, and necrotic foci. Because MERRF is characterized by neuronal loss, astrocytosis, and degeneration of myelinated tracts[12], it seems that EP studies may only reflect the severity of mitochondrial dysfunction in part. Abnormal EPs indicate that the myelin has been involved by the mitochondrial disease, though it is not the primary lesion. Therefore, for mitochondrial disease, EP is an adjunct of methods to detect the functional disturbance of the nervous system and to screen the distribution of the involvement.

ACKNOWLEDGEMENT

The study was in part presented in the 8th International Evoked Potentials Symposium in Fukuoka, Japan in 2004; and was in part supported by Taipei VGH grant (93-282) and NSC grant (94-2314-B-075-022).

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