ORIGINAL ARTICLE

Different Phenotypes Caused by the Unique Mutation in the Same Family with Mitochondrial Encephalomyopathy

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ABSTRACT

Background and Objective: Mitochondrial encephalomyopathies represent a clinically heterogeneous group of disorders resulting from abnormal mitochondrial function. This study investigates the clinical and genetic characteristics of families with mitochondrial encephalomyopathy.

Methods: The clinical manifestations, biopsy and gene detection were retrospectively analyzed for four probands with definitively diagnosed mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) from three families with MELAS and/or maternally inherited diabetes and deafness (MIDD).

Results: The initial symptoms of probands were convulsive headache and/or epilepsy. The members of the three families also had diabetes, deafness, muscle weakness and short statures. Typical characteristics were indicated by muscle biopsy and gene detection in all.

Conclusion: We reveal that the same family can have MIDD and MELAS cases, which clearly show that the unique mutation may cause different syndromes in one family. Neurologists should take into account more possibilities and phenotypes in screening and genetic counselling for the families of probands.

KEYWORDS: Mitochondrial encephalomyopathy, MELAS syndrome, MIDD syndrome, A3243G point mutation, Ragged red fibers.

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INTRODUCTION

Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) and maternally inherited diabetes and deafness (MIDD) are distinct syndromes, but both of them are caused by the same mutation of mitochondrial DNA, and are different phenotypes of mitochondrial encephalomyopathy.[1-4] Ragged red fibers (RRF) in muscle biopsy, mtDNA point mutation from gene detection, and the absence of fragment and decreasing copy number are helpful for definitive diagnosis of MELAS.[3,4] The early symptoms of mitochondrial encephalomyopathy are non-specific, the clinical manifestations of mitochondrial encephalomyopathy are complicated and various, so the early diagnosis is very difficult.[1,4] We retrospectively analyzed the clinical manifestations,

lactic acid levels, pathology, genotypes and other aspects of patients and members of their family, in order to accumulate clinical researching data, and to find shared characteristics of the diseases.

METHODS

The definitive diagnoses of MELAS and MIDD cases and from the Department of Neurology in the Zhenjiang Fourth Hospital Affiliated to Jiangsu University and the family members of the cases from August 16, 2010 to July 16, 2018 were included in this study after providing written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Review Board of Jiangsu University (Clinical trial registration number: Zj20100701007). The clinical manifestations, laboratory findings, imaging (brain CT and MRI, MRS), characteristics of muscle biopsy and gene detection of the point mutations of the 10 cases (1 male, 9 females, the minimal age: 9 years, the maximal age: 37 years) were gathered. Three inherited families of the four patients (two probands were from the same family) were engaged for the study. Medical history, lactic acid levels, imaging, genotypes, and muscle biopsies of the three families were screened.

Muscle Biopsy Analysis

Muscle biopsies were frozen in liquid nitrogen. Cryostat sections were performed from the routine examination of the reaction, according to reported procedures. The frequency of deficient muscle fibers on cytochrome c oxidase (COX) stain; and of RRF on modified Gomori trichrome (MGT) and succinate dehydrogenase (SDH) stains were determined by counting the muscle fibers of each specimen.

DNA extraction and molecular genetic analysis

The mtDNA3243 A > G were examined by polymerase chain reaction (PCR) and direct sequencing. For sequencing, a Perkin Elmer Big Dye Sequencing kit (Perkin-Elmer, Shelton, CT, USA) and an AB 3130 sequencer (Applied Biosystems, USA) were used. Four common mutation loci A3243G, A8344G, T3271C and T8993C were detected using the urine of the patients in this experiment.

RESULTS

Pedigree of the Families

The pedigree of the families carrying the mitochondrial DNA A3243G mutation is shown in Figure-1. The clinical manifestations of the members from the families are shown in Table-1.

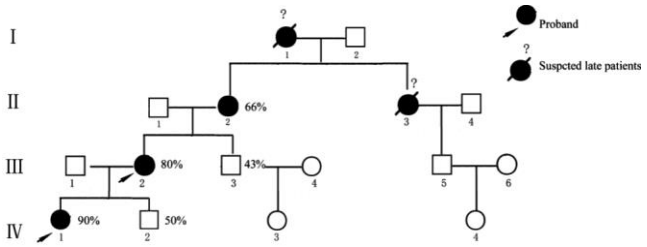

Fig.1: Pedigree of the first family the number to the right of the symbols displays heteroplasmy levels.

Clinical Manifestations

The comparisons of the clinical manifestations among the de novo MELAS, probands and other family members are shown in the Table-1, the conditions about the clinical manifestations of the different families are different. Although the clinical manifestations of MELAS are heterogeneous, initial symptoms of the probands are very typical, and so are the de novo and other family members. All the patients had epilepsy, and all the probands experienced convulsive headache at the beginning. Muscle weakness, mental retardation and stroke-like symptoms were seen in most of the patients. Diabetes (12/22) and hearing impairment (6/22) were also common in the families.

Imaging

T1WI of MRI in Figure 2 indicated a low signal, T2WI, FLAIR and DWI sequence indicated a high signal. MRS in 3 cases indicated N-acetyl aspartic acid (NAA) was decreased and lactic acid was increased in the lesions, but there was no lactate peak. Five cases had different levels of brain atrophy，furthermore, two cases had calcification. Additional MRI CT of one patient with MELAS was performed at a different time (Fig.2).

Table-1: The characteristics of cases in the families.

Family	Generation	Sex	Age (y)	Height (cm)	Weight (kg)	Seizure	Deafness	Myotrophy	Diabetes	Other symptoms	Lactic Acid (mmol/L)	Mutation rate	Biopsy	Diagnosis
1	II2	F	63	157	45 (thin)	-	From 43y	Mild	From 43y	-	-	66%	-	MIDD
	II3	F	Dead	-		-	From 30y	-	From 28y	-	Died from ketoacidosis	-	-	MIDD
	III2*	F	39	161	49	-	From 34y	-	From 24y	Pancreatitis from 38y, Weakness, Blurred vision	2.8(0.7-2.7 mmol/L)	80%	High-contracted fiber (HE,MGT,COX&SDH)	MELAS and MIDD
	III3	M	36	183	75	-	-	-	-	-	-	43%		
	IV1*	F	15	153	37.5 (thin)	+	+	+	From 11y	Stroke-like symptoms, short, hairy, seizure, headache, vomiting,	8.2 (0.7-2.7 mmol/L)	90%	RRF(HE), High-contracted fiber (HE, MGT, COX&SDH)	MELAS and MIDD
	IV2	M	9	135	32.5	-	-	-	-	-	-	50%	-	
2	I1	F	Dead at 70y	158	N/A	-	From 40y	-	From 36y	NA	NA	38%	N/A	MIDD
	II3	F	48y	156	N/A	-	From 37y	-	From 33y	-	-	-	N/A	MIDD
	III2	F	45	156	50	From 28y	-	-	From 24y	Headache, weakness Sensory aphasia, gibberish	5.8(0.7-2.7 mmol/L)	78%	RRF(HE,MGT), APM (HE,MGT,COX&SDH), increased MC? (SDH)	MELAS
	III3	F	40	158	52	-	From 29y	-	-		-		-	
	IV1*	F	21	161	55	From 18y	-	Mild	From 11y	Headache, weakness	6.6 (0.7-2.7 mmol/L)	82%	RRF(HE,MGT), APM r(HE, MGT, COX&SDH), increased MC? (SDH)	MELAS
3	II2	F	68	134	N/A	From 29y	From 35y	N/A	From 40y	dwarfish	-	N/A	N/A	MIDD
	II3	F	64	156		-	-		From 38y	Headache, weakness Sensory aphasia, gibberish	5.8(0.7-2.7 mmol/L)		N/A	MIDD
	II5	M	58	N/A		-	From 36y	N/A	From 42y		-		N/A	MIDD
	III1*	F	Dead at 18y	146	32	From 8y	From 12y	N/A	From 11y	Headache, vomiting, paralysis, phonism, childish, dwarfish	2.55 (0.7-2.7 mmol/L)	86%	RRF(HE,MGT), APM r(HE,MGT,COX&SDH), increased MC? (SDH)	MELAS and MIDD

*Probands

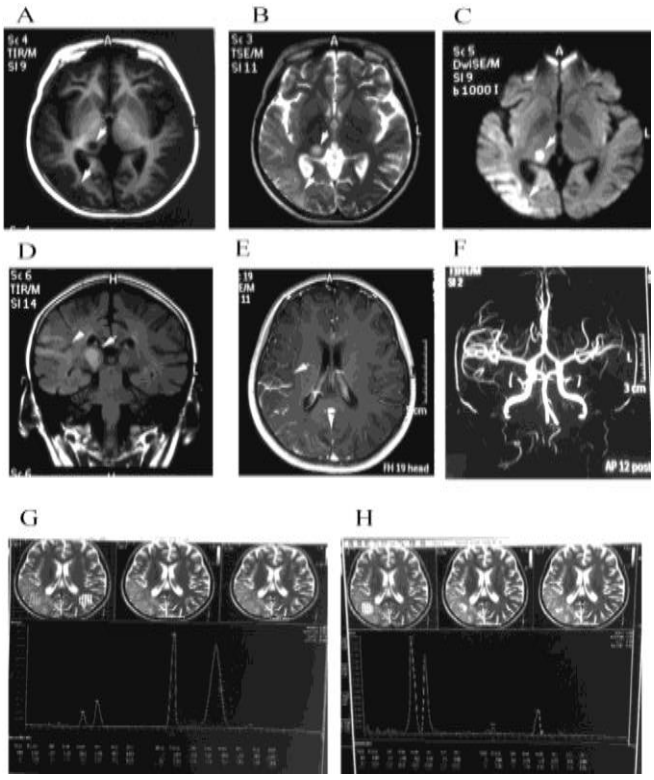

Fig.2: MRI, MRS, MRI enhanced MRI and MRA acute phase images. **A:** T1W1 showed low-signal density in the cortex and subcortex of the right thalamus, parietal, occipital and temporal lobes. **B:** T2W1 showed high-signal density. **C:** DW1 showed high-signal density. **D:** Flair showed high-signal density. **E:** Enhanced MRI showed gyri-like strengthening. **F:** MRA didn't show abnormal lesions. **G:** MRS indicated that NAA was normal and lactic acid had increased in the normal tissue on the next hemisphere to the lesions. **H:** MRS indicated that NAA had decreased and lactic acid had increased.

EEG

There were high, sharp abnormal waves in the left hemisphere in Case 4 and slowed, diffuse inactive echo waves in the other 5 cases.

Muscle biopsy

Muscle biopsy pathology was detected in 7 cases and 2 families. Haematoxylin Eosin and MGT indicated ragged red fibers in 5 cases (including Case 1 and 2 without mutation detected); SDH enzyme staining revealed the enhanced enzyme activity of mitochondrial complex II; anti-mitochondrial antibody staining showed abnormal proliferation of mitochondria (Fig. 3).

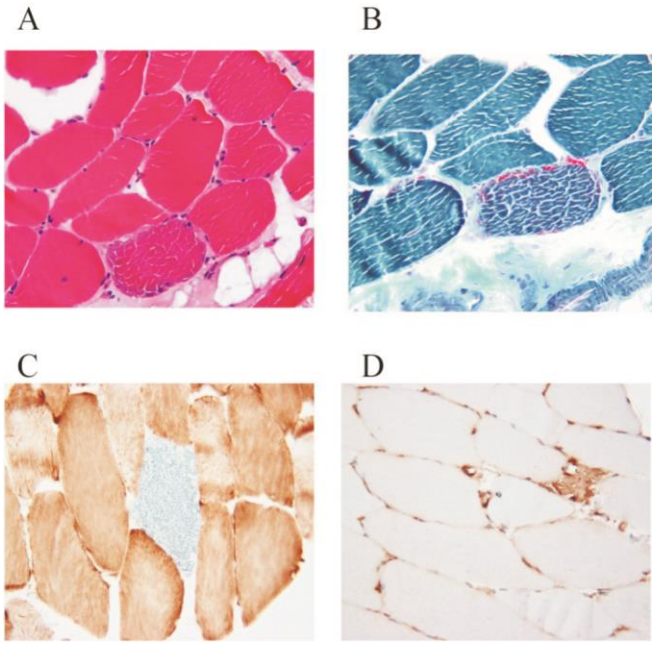

Fig.3: A. RRF with a red border is shown (Gomori stain×400). **B.** RRF with purple kytoplasm is shown (HE stain×200). **C.** Blue RRF is shown (COX and SDH stain×200). D. Brown fibers are shown indicating the abnormal proliferation of mitochondria (Anti-mitochondrion immunohistochemistry stain×400). Abbr: COX: cytochrome c oxidase; MGT: modified Gomoritrichrome: RRF: Ragged red fibers; SDH: succinate dehydrogenase.

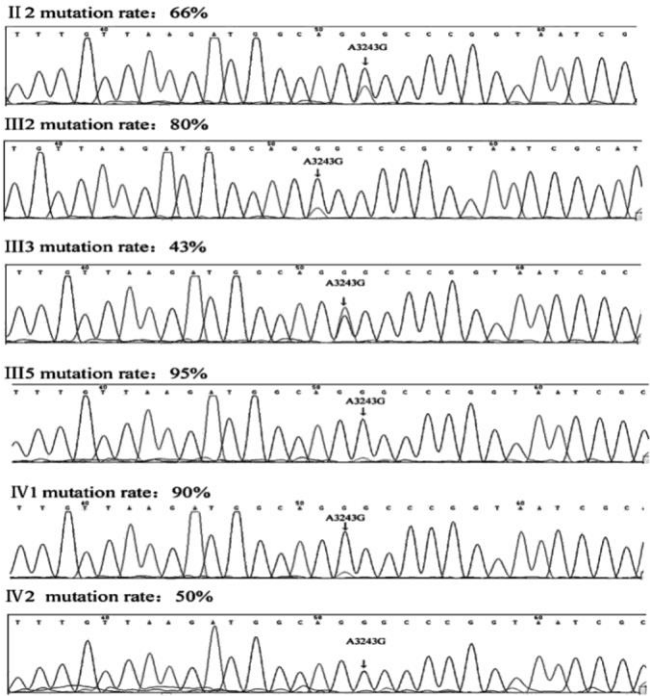

Fig.4: Results of gene detection of the family Red arrow means the mutation point.

Gene Detection

Common mutation loci A3243G were detected using the urine of the patients in all probands and "de novo" MELAS. The base A (adenine) to G (guanine) substitution mutation takes place in DNA loci 3243 of mitochondrial DNA (Mutation rate are in Table-1 and Fig. 4).

DISCUSSION

Encephalomyopathy are rare and diverse, and are difficult to diagnose for neurologists. In this study seizures and headaches clearly manifest earlier than the lesions in imaging, which should be used as an important indicator of early diagnosis. Furthermore, diabetes and hearing impairments are common manifestations of mitochondrial encephalomyopathy, which often have familial incidence, as about one third of the members in the families had MIDD. According to above results, there are 3 cases with both MELAS and MIDD, 7 cases with MIDD, and there are 2 cases with MELAS (Table-1).

It is difficult to discern MELAS and MIDD totally in the same family in practice. MELAS and MIDD in our study were overlapping, it is reported some MIDD could evolve into MELAS over long time, that is to say that MELAS and MIDD are two stages of the same disease in certain case, but there are no other cases to support it, and more studies should be carried out to reveal the relations between them. Patients with de novo MELAS in this study shown similar symptoms with the probands, which is a coincidence with the reports.[5,6] But the differences between the family members and the probands were distinct, which may be caused by different mutation rates. The family members had mainly been affected by MIDD with low mutation rates, while probands had predominantly more lesions in the brain with high mutation rates, which were more acute and severe, and were in coincidence with the reports that low levels of the mitochondrial tRNALeu (UUR) 3243A > G mutation can cause MIDD.[7] Why some patients showed MELAS and some MIDD is unknown. It might be related to individual differences.

The clinical manifestations caused by the same mutation are still heterogeneous and diverse, so an early clinical diagnosis is difficult. Serum lactic acid and imaging can be detected combined with medical and family history. If there are positive results, then histology and genetic examination should be carried out, which are necessary to make a definitive diagnosis.[7-9]

The patients' family screening can confirm asymptomatic carriers with mild symptoms, and it is helpful in providing genetic counseling and complete corresponding defense measures. Family screening can be used as the conventional treatment process for mitochondrial diseases.

CONCLUSION

MELAS and MIDD are overlapping and different phenotypes of encephalomyopathy. Seizures and headaches should be used as an important indicator of early diagnosis. Diabetes and hearing impairments are common manifestations of mitochondrial encephalomyopathy, and often have familial incidence. Doctors should take into account more possibilities and phenotypes in screening and genetic counselling for the families of probands.

LIMITATIONS OF THE STUDY

The study did not include the patients' family screening for asymptomatic carriers with mild symptoms. As it is helpful in providing genetic counseling and complete corresponding defense measures for the conventional treatment process for mitochondrial diseases. Hence more studies should be carried out to strengthen the results and reveal the relation between different phenotypes of encephalomyopathy.

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CONFLICT OF INTEREST

None to declare.

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REFERENCES

1. Kurt YG, Kurt B, Aydin I, Agilli M, Aydin FN. NFU1 gene mutation and mitochondrial disorders. Neurol India. 2016; 64 (7): 630-2.
2. Cardenas-Robledo S, Saber Tehrani A, Blume G, Kattah JC. Visual, ocular motor, and cochleo-vestibular loss in patients with heteroplasmic, maternally-inherited diabetes mellitus and deafness (MIDD), 3243 transfer RNA mutation. J Neuroophthalmol. 2016; 36 (2): 134-40.
3. Yee ML, Wong R, Datta M, Fazlo TN, Ebrahim MM, Mcnamara EC, et al. Mitochondrial disease: an uncommon but important cause of diabetes mellitus. Endocrinol Diabetes Metab Case Rep. 2018;2018(1): 18-91.
4. Mancuso M, Orsucci D, Angelini C, Bertini E, Carelli V, Comi GP, et al. The m.3243A > G mitochondrial DNA mutation and related phenotypes. A matter of gender? J Neurol. 2014; 261 (12): 504-10.
5. Laat P, Janssen MC, Alston CL, Taylor RW, Rodenburg RJ, Smeitink JA. Three families with 'de novo' m.3243A > G mutation. BBA Clin. 2016; 29 (4): 19-24.
6. Nesbitt V, Pitceathly RD, Turnbull DM, Taylor RW, Sweeney MG, Mudanohwo EE, et al. The UK MRC mitochondrial disease patient cohort study: clinical phenotypes associated with the m.3243A > G mutation-implications for diagnosis and management. J Neurol Neurosurg Psych. 2013; 84 (1): 936-38.
7. Procaccio V, Neckelmann N, Paquis-Flucklinger V, Bannwarth S, Jimenez R, Davila A, et al. Detection of low levels of the mitochondrial tRNALeu (UUR) 3243A > G mutation in blood derived from patients with diabetes. Mol Diagn Ther. 2006; 10 (6): 381-9.
8. Laat P, Koene S, van den Heuvel LP, Rodenburg RJ, Janssen MC, Smeitink JA. Clinical features and heteroplasmy in blood, urine and saliva in 34 Dutch families carrying the m.3243A > G mutation. J Inherit Metab Dis. 2012; 35 (111): 1059-69.
9. Liu F, Zhang D, Yan C. Expanding the clinical spectrum of myopathy, encephalopathy, lactic acidosis, and stroke-like (MELAS) episode: A case with A3243G mitochondrial DNA mutation presenting as MELAS and congenital melanocytic naevi overlap. Neurol India. 2016; 64 (3): 336-8.

Author's Contribution

XHP MR, YM: Conception and design of study, revised the manuscript with intellectual input.

NFN: Acquisition of data and immunohistochemical staining and gene.

XHP, ZWF, JB, ZKR: Acquisition of data. Drafting the manuscript and critical revision.

ALL AUTHORS: Approval of the final version of the manuscript to be published.