

Clinical and Molecular Characteristics in 100 Chinese Pediatric Patients with m.3243A>G Mutation in Mitochondrial DNA

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Abstract

Background: Mitochondrial diseases are a group of energy metabolic disorders with multisystem involvements. Variable clinical features present a major challenge in pediatric diagnoses. We summarized the clinical spectrum of m.3243A>G mutation in Chinese pediatric patients, to define the common clinical manifestations and study the correlation between heteroplasmic degree of the mutation and clinical severity of the disease.

Methods: Clinical data of one-hundred pediatric patients with symptomatic mitochondrial disease harboring m.3243A>G mutation from 2007 to 2013 were retrospectively reviewed. Detection of m.3243A>G mutation ratio was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism. Correlation between m.3243A>G mutation ratio and age was evaluated. The differences in clinical symptom frequency of patients with low, middle, and high levels of mutation ratio were analyzed by Chi-square test.

Results: Sixty-six patients (66%) had suffered a delayed diagnosis for an average of 2 years. The most frequent symptoms were seizures (76%), short stature (73%), elevated plasma lactate (70%), abnormal magnetic resonance imaging/computed tomography (MRI/CT) changes (68%), vomiting (55%), decreased vision (52%), headache (50%), and muscle weakness (48%). The mutation ratio was correlated negatively with onset age ($r = -0.470$, $P < 0.001$). Myopathy was more frequent in patients with a high level of mutation ratio. However, patients with a low or middle level of m.3243A>G mutation ratio were more likely to suffer hearing loss, decreased vision, and gastrointestinal disturbance than patients with a high level of mutation ratio.

Conclusions: Our study showed that half of Chinese pediatric patients with m.3243A>G mutation presented seizures, short stature, abnormal MRI/CT changes, elevated plasma lactate, vomiting, and headache. Pediatric patients with these recurrent symptoms should be considered for screening m.3243A>G mutation. Clinical manifestations and laboratory abnormalities should be carefully monitored in patients with this point mutation.

Key words: Clinical Symptom; Heteroplasmy; Mitochondrial A3243G Mutation; Mitochondrial Disease

INTRODUCTION

Mitochondrial diseases are a group of energy metabolism disorders with multisystem involvement. They can be caused by mutations in nuclear genes either relating to mitochondrial components or mitochondrial DNA (mtDNA). The prevalence of mitochondrial diseases due to mutations in mtDNA and related nuclear genes is estimated to be 1 in 5000.^[1] Mutations in mtDNA are much higher than those in the related nuclear genes, probably because that mitochondrial genome has been exposed in reactive oxygen species (ROS) and lacked protection structure.

In 1990, the adenine to guanine transition at the mtDNA position of 3243 encoding tRNA^{Leu (UUR)} was found to be the molecular basis for mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS).^[2,3] Epidemiological studies showed that m.3243A>G mutation

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is the most frequent pathogenic mutation in mtDNA. The prevalence of m.3243A>G mutation in mtDNA is 3.5/100,000 adults in the North East of England^[4] and approximately 1/6000 in an adult population in Finland.^[5] The clinical features of m.3243A>G mutation are highly variable.^[6-8] Seizures, encephalopathy, and stroke-like episodes were found in about 80% patients with MELAS, and short stature, deafness, cognitive impairment, exercise intolerance, migraines, depression, cardiomyopathy, cardiac conduction defects, and diabetes mellitus were also found in some MELAS cases.

Here, we summarized the clinical spectrum of m.3243A>G mutation in Chinese pediatric patients, to define the common clinical manifestations and study the correlation between heteroplasmic degree of the mutation and clinical severity of the disease.

METHODS

Subjects

Clinical data of 100 Chinese pediatric patients who were first diagnosed as mitochondrial diseases through gene test for m.3243A>G mutation in Peking University First Hospital from 2007 to 2013 were retrospectively reviewed. Informed consent was obtained from patient or patient's guardian. Patients without complete clinical information and laboratory data were excluded. This study was approved by the Medical Ethics Committee of Peking University First Hospital (No. 2009 (23)).

A total of ten clinical characteristics including vision impairment, hearing loss, encephalopathy, myopathy, and gastrointestinal disturbances were collected from these patients. Short stature was defined as the body height less than two standard deviations below the mean height of normal children. Myopathy was manifested as exercise intolerance, weakness, gait instability, etc. Exercise intolerance was defined as poor endurance in sports.

Mitochondrial DNA analysis

The peripheral blood samples were collected from these patients when they were first screened for the gene mutation. Total DNA was extracted from blood sample using a routine method.^[9] Detection of m.3243A>G mutation ratio was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism method. The PCR product was digested with restriction enzyme Apa I, and then separated in 2% agarose gel. Finally, the density of electrophoretic band was measured and calculated for mutation ratio.^[10] The patients were divided into three groups based on mutation ratio (low level: 0–30%; middle level: 31–60%; and high level: 61–100%).

Statistic analysis

SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The normality of quantitative data was tested by Kolmogorov-Smirnov test. Nonparametric test was used if the data did not conform to Gaussian distribution. Age

and mutation ratio inconsistent with Gaussian distribution were presented as median (Q_1 , Q_3). Correlation between m.3243A>G mutation ratio and age was evaluated with Spearman's rank correlation method. The differences in clinical symptom frequency of patients with low, middle, and high levels of mutation ratio were analyzed by Chi-square test. A $P < 0.05$ was considered statistically significant.

RESULTS

The age of the diagnosis of mitochondrial disease ranged from 2 months to 18 years, with the median age of 9 years (5.8 years, 12.0 years). The median onset age was 7 years (4.0 years, 9.0 years). A delayed diagnosis for an average of 2 years was found in 66% patients. Forty patients were males and sixty were females (ratio = 1.0:1.5). The m.3243A>G mutation ratio varied from 5% to 93%, with a median level of 44% (36%, 54%). There was no significant difference in m.3243A>G mutation ratio between males and females ($t = 0.691$, $P = 0.491$).

Clinical features of patients

Patients at the onset had one or more symptoms, including seizures (54%), muscle weakness (29%), headache complicated with vomiting (25%), decreased vision (18%), hearing loss (10%), impaired verbal communication (6%), and heart preexcitation syndrome (5%). The most prevalent symptom of these patients was seizures (76%), followed by short stature (73%), elevated plasma lactate (70%), abnormal magnetic resonance imaging/computed tomography (MRI/CT) changes (68%), vomiting (55%), decreased vision (52%), headache (50%), and muscle weakness (48%). Most of the patients were multisymptomatic, only two patients had one symptom, and five patients manifested two symptoms.

Seizures were present in 76 patients (76%), of which 13 patients had stroke during or shortly after seizures. Recurrent headaches were found in 50 patients (50%), of which most were complicated with vomiting and/or vision loss. Sixty-eight patients (68%) were found to have MRI/CT abnormalities, including abnormal asymmetric signals in occipital area (51/68, 75%), temporal area (21/68, 31%), parietal area (20/68, 29%), bilateral basal ganglia calcification (16/68, 24%), cerebral atrophy (10/68, 15%), and thalamus and brainstem lesions (5/68, 7%). Twenty-one patients (21%) had verbal communication difficulties. Slurred speech was present in 16 patients, of whom 3 had progressively worsening speech, and 2 had delayed speech development (at 1.5 and 3.0 years of age).

Growth impairment was found on height or/and weight in 77 patients. Seventy-three patients (73%) had a short stature and 69 patients (69%) had a weight loss. Gastrointestinal disturbance was also a common symptom for m.3243A>G patients. Fifty-five patients (55%) had experienced recurrent vomiting and 38 patients (38%) had diarrhea or/and constipation.

Plasma lactate ranged from 1.4 to 19.0 mmol/L (normal range: 0.7–2.0 mmol/L). Elevated plasma lactate was

detected in 70 patients (70%), of which 90% had a plasma lactate >5 mmol/L. Hyperglycemia (>6.1 mmol/L) was found in ten patients (10%).

Vision impairment was found in 52 patients (52%), of which 33 experienced blurred vision and 16 had decreased visual acuity. Failure to track object or light with their eyes was detected in 3 infants.

Reduced muscle strength was reported in 48 patients (48%), of which 31 had minimal muscle weakness in upper limbs and shoulders and 17 manifested muscle weakness in lower limbs, and 2 had walk difficulties. Thirty-seven patients (37%) complained of difficulties in maintaining stability, of them 18 experienced frequent tumbling, and 38 patients (38%) had compromised exercise tolerance, which appeared during running or going upstairs in most cases and during walk on flat places in only two cases. Eighteen patients (18%) had bilateral ptosis.

Heart diseases were detected in 35 patients (35%), of which 17 were found to have abnormal electrocardiogram such as ST-T changes and arrhythmias. Left ventricular hypertrophy was found in eight patients, right ventricular hypertrophy in five patients, and preexcitation syndrome in five patients.

Twenty-one patients (21%) had hearing impairment, presenting tinnitus or hearing loss (mild deafness in 14 patients, moderate deafness in 5 patients, and severe deafness in 2 patients).

Correlation between m.3243A>G mutation ratio and onset age

The m.3243A>G mutation ratio in peripheral leukocytes was determined in all the patients, and 32% of them had a mutation ratio above 50%. The relationship between m.3243A>G mutation ratio and onset age was analyzed by Spearman's rank correlation method, which showed that m.3243A>G mutation ratio was correlated negatively with onset age [$r = -0.470$, $P < 0.001$; Figure 1].

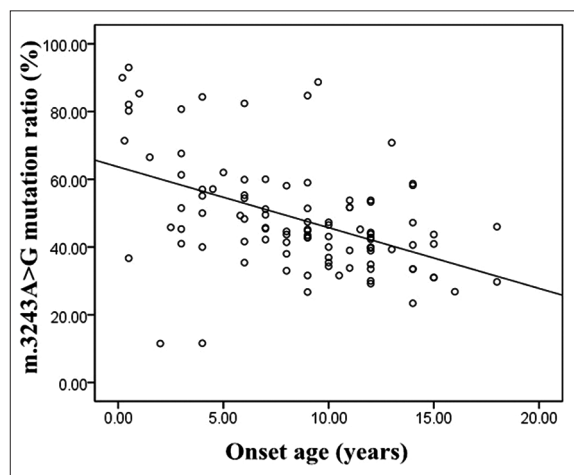


Figure 1: Correlation between m.3243A>G mutation ratio in peripheral leukocytes and onset age ($r = -0.470$, $n = 100$).

Clinical symptom frequency of patients with different mutation ratio

The differences in clinical symptom frequency among patients with low, middle, and high levels of mutation ratio were analyzed by Chi-square test [Table 1]. There were significant differences in the frequencies of hearing loss, decreased vision, myopathy, and gastrointestinal disturbance among three groups. Myopathy was more frequent in patients with a high level of mutation ratio. However, patients with a low or middle level of m.3243A>G mutation ratio were more likely to suffer from hearing loss, decreased vision, and gastrointestinal disturbance than high level group.

DISCUSSION

The m.3243A>G mutation has been shown to lead to reduced levels of the tRNA^{Leu (UUR)}, decrease in aminoacylation, and absence of the normal modification with 5-taurinomethyl group at the wobble base.^[11,12] Mitochondrial disease caused by this mutation may result from the reduction of mtDNA-encoded proteins and oxidative phosphorylation activity in mitochondrial translation. The m.3243A>G mutation is usually present as heteroplasmic state.^[13] Its phenotype is highly variable, ranging from asymptomatic to mild or severe phenotype.^[14] Several clinical syndromes including MELAS, myoclonic epilepsy with ragged-red fibers, chronic progressive external ophthalmoplegia, and Leigh's syndrome may associate with m.3243A>G mutation.^[6,15-18] Patients with m.3243A>G mutation but without clinical symptoms are not uncommon.^[19] To define the spectrum of clinical manifestations due to m.3243A>G mutation, we retrospectively reviewed clinical data of 100 Chinese pediatric patients with m.3243A>G mutation regardless of their clinical presentations.

Central nervous system (CNS) is frequently involved in mitochondrial diseases because of its higher energy demand. Seizures may result from neuronal energy depletion, oxidative stress, impaired calcium signaling, immune disturbances, and deficiencies of vitamins, cofactors, and amino acids.^[20] In this study, the CNS symptoms of seizures and headache were found in more than 50% patients. The prevalence of seizures was as high as 76%, and was nearly 100% in our previous study,^[21] similar to the prevalence of 70.5% in a report of pediatric mitochondrial disease from Taiwan, China.^[22] Brain consumes more energy in children than adults. CNS symptoms are, therefore, more common and severe in children than adults. The prevalence of seizures ranged from 9% to 20% in adults.^[23,24] In MELAS patients, recurrent stroke-like episodes were more frequent in those with m.3243A>G mutation than those without the mutation.^[25,26] We found stroke-like episodes in 22% patients, higher than the prevalence from patients with mitochondrial mutations other than m.3243A>G mutation.^[22]

Most of our patients showed abnormal brain image findings were probably not due to vascular injuries. Bilateral basal ganglia were the most vulnerable site in this disease, followed by temporal, parietal, and occipital area, similar

Table 1: Analysis of clinical symptom frequency in different distributions of 3243A>G mutation ratio, n

Clinical symptom frequencies	Low level of mutation ratio (n = 8)	Middle level of mutation ratio (n = 75)	High level of mutation ratio (n = 17)	χ^2	P
Decreased vision	3	45	4	6.423	0.040
Hearing loss	3	18	0	6.239	0.044
Encephalopathy	5	63	13	2.444	0.295
Gastrointestinal disturbance	6	56	5	13.089	0.001
Diabetes	1	8	1	0.413	0.813
Growth impairment	6	59	12	0.530	0.767
Myopathy	4	56	16	6.097	0.047
Heart disease	4	26	5	1.028	0.598
MRI/CT changes	5	52	11	0.257	0.879
Elevated lactate	5	55	10	1.622	0.444

Low level: A mutation ratio range 0–30%; Middle level: A mutation ratio range 31–60%; High level: A mutation ratio range 61–100%; MRI: Magnetic resonance imaging; CT: Computed tomography.

to the previous report.^[27] Chae *et al.*^[25] also observed the tendency that basal ganglia were frequently involved in patients without m.3243A>G mutation.

Gastrointestinal symptoms were common (67%) in this series of patients. Vomiting was more frequent than constipation or/and diarrhea, and 68% vomiting cases were complicated with headaches. A higher prevalence (42%) of these symptoms was also reported in a recent study.^[24] Therefore, mitochondrial diseases should be considered clinically in children with unexplained vomiting and headaches.

The prevalence of cardiac involvement was highly variable in patients with mtDNA mutations, ranging from 17% to 40%.^[28,29] In this study, 35% patients exhibited cardiac involvement with the main manifestations of abnormal electrocardiogram (17%) and left ventricular hypertrophy (8%). The prevalence of left ventricular hypertrophy was less in our patients than the patients with m.3243A>G mutation (56%) reported by Majamaa-Voltti *et al.*^[30] Pediatric patients may be the reason of that lower prevalence of cardiac involvement was present in this study. For patients suspected of mitochondrial disease, routine electrocardiogram, and ultrasonocardiography should be performed.

The m.3243A>G mutation ratio in peripheral leukocytes was negatively correlated with patients' onset age. Previous studies have also described a decrease of mutant mtDNA with increasing age.^[21,31] Prospective studies are needed to confirm this phenomenon. Among these symptoms we described, myopathy was frequently seen in patients with high level of m.3243A>G mutation ratio. However, patients with a low or middle level of m.3243A>G mutation ratio were more likely to suffer from hearing loss, decreased vision, and gastrointestinal disturbance.

In conclusion, this study summarized the common symptoms and onset symptoms in a large cohort of Chinese pediatric patients with m.3243A>G mutation. In view of the fact that the diagnosis of 66% of patients was delayed an average of 2 years, we suggested that examination of m.3243A>G mutation in mtDNA should be considered in children with one or more of the symptoms including seizures, short

stature, muscle weakness, headache complicated with vomiting, decreased vision, and hearing loss. Although the mutation ratio in blood sample is an available test for diagnosis of mitochondrial disease, the m.3243A>G mutation ratio is usually higher in muscle and urine sample. Therefore, the mutation ratio in other tissues, such as muscle and urine cell, should be included in the future study.

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Conflicts of interest

There are no conflicts of interest.

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