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Clinical, biochemical, and genetic analysis of the mitochondrial respiratory chain complex I deficiency

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Abstract

Mitochondrial respiratory chain complex I deficiency is one of common mitochondrial disorders. However, the information is relatively little about the features of Chinese patients. In this study, the clinical, biological, and genetic analyses were performed in the children with respiratory chain complex I deficiency, in order to further understand the characteristics of the disease.

Over a 3-year period, 67 patients (37 boys, 30 girls), presenting with unexplained multisystemic symptoms and signs were recruited. Clinical and laboratory data of the patients were summarized. Spectrophotometric assay was used for the analysis of mitochondrial complex I-V enzyme activity in peripheral leukocytes. The entire mitochondrial DNA (mtDNA) sequence was analysed for patients and their mothers.

The children with respiratory chain complex I deficiency presented with multisystem dysfunction. Onset occurred before the third year of life in 96.9% patients without mtDNA mutation. Onset occurred before the third year of life in 76.5% of patients with mtDNA mutation ($P=.03$). About 51.5% of patients without mtDNA mutation had weakness, which is higher than 24% patients with mtDNA mutation ($P=.02$). Isolated complex I deficiency and combined complex I deficiency were found in 45 and 22 patients, respectively. The prevalence of isolated complex I deficiency was higher in the patients with mtDNA mutations (79.4%) than in the patients without mtDNA mutations (54.5%).

Patients with nuclear DNA mutations are more likely to develop early onset in mitochondrial respiratory chain complex I deficiency. The patients with complex I deficiency of peripheral leukocytes may be more likely to be caused by mtDNA mutation.

Abbreviations: mtDNA = mitochondrial DNA, OXPHOS = oxidative phosphorylation, PCR = polymerase chain reaction.

Keywords: electron transport, mitochondrial diseases, mitochondrial DNA, respiratory chain complex I deficiency

1. Introduction

Mitochondria hold a central position in cellular oxidative phosphorylation (OXPHOS). Mitochondrial OXPHOS produces amounts of ATP for cellular requirements. The respiratory chain complex I (Nicotinamide adenine dinucleotide -ubiquinone oxidoreductase EC 1.6.5.3) is not only the point of respiratory chain but also the largest and most complicated complex, containing 45 subunits, 7 encoded by mitochondrial DNA (mtDNA) and 38 by nuclear DNA. Mitochondrial diseases due to a reduced capacity for OXPHOS are recognized to be significant. The incidence of mitochondrial diseases due to a reduced capacity for complex I is now recognized to be quite significant. Isolated complex I deficiency is the most commonly identified biochemical defect in childhood-onset mitochondrial disease, accounting for approximately one-third of all cases of OXPHOS disorders.[1] Respiratory chain complex I deficiency is associated with several neurological disorders. There is little reported information about mitochondrial diseases caused by complex I deficiency in China, due to a lack of clinical assays for respiratory chain enzymes. The associated clinical and genetic heterogeneity leads to considerable diagnostic challenges. In order to know well the characters of Chinese patients with respiratory chain complex I deficiency, further studies were performed on the basis of our previous researches.

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2. Materials and methods

2.1. Patients

In the past 3 years, 67 children with suspected mitochondrial disorders, ranging from 20 days to 15 years, were recruited. The male: female ratio was 1.23 (37: 30). These patients presented with unexplained symptoms and signs, including motor

developmental delay or regression, delay or regression in mental development, weakness, cardiomyopathy, kidney dysfunction, and liver dysfunction that were progressively worsening. Typical aminoacidopathies, organic acidurias, and mitochontrial β-oxidation defects were excluded by blood amino acids, acylcarnitines, and urinary organic acid analysis. These patients were non-consanguineous. The parents of all patients were informed about the research, and they consented to participate. This study was approved by the hospital ethics committee.

2.2. Assays for mitochondrial respiratory chain complexes I to V

Peripheral leukocytes and mitochondria were sequentially isolated from 5 mL of anticoagulated venous blood, as previously described.[2] In brief, 5 mL blood was added to 50 mL of a lysis buffer and left standing for 30 minutes. The resultant homogenate was centrifuged for 10 minutes at 4000*g*, and the precipitate was resuspended in the lysis buffer. Highly purified leukocytes were collected by repeating the above procedure 3 times. The leukocytes were washed and then suspended in phosphate-buffered saline. The homogenate thus obtained was centrifuged for 20 minutes at 600*g* at 4 °C. Next, the mitochondria-rich supernatant was collected and centrifuged for 10 minutes at 11,000*g* at 4 °C, and mitochondrial pellets were obtained.

The activities of mitochondrial respiratory chain enzymes I to V were measured using spectrophotometry. The rate of citrate synthase, a mitochondrial matrix enzyme, was measured to determine the integrity of the mitochondrial preparation. Therefore, the activity of each complex is expressed both as a rate (nmol/min/mg of mitochondrial protein) and as a ratio of the rate of citrate synthase, as previously described. Eighty-two normal children were used as normal controls.

2.3. Mitochondrial gene sequence analysis

Total DNA was extracted from the blood, using conventional phenol/chloroform protocols. The entire sequence of the mitochondrial genomes of 67 patients and their mother was amplified by polymerase chain reaction (PCR) into 8 overlapping fragments, using sets of light- and heavy-strand oligonucleotide primers. Each fragment was purified and analyzed by direct sequencing.[3] The direct sequencing of the PCR product was completed by the company (TIANGEN, Beijing, China). The sequence results were then compared with the revised Cambridge reference sequence (http://www.mitomap.org), GenBank ID: NC_012920.1.

PCR was performed in a 25-μL flask containing 50 ng of DNA, the PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 μM of each primer, and 1.25 U Taq DNA polymerase. The reaction was carried out with an initial denaturation step for 5 minutes at 95 °C, followed by 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds, and a final elongation step for 4 minutes at 72°C.

2.4. Statistical analysis

All data were analyzed using the SPSS 13.0 software, and $P < .05$ was considered statistically significant. Data are expressed as the mean ± SD for normal distributions and as median and interquartile range for non-normal distributions. The Student *t* test for independent groups was used to compare means, and the χ^2 test was used to compare proportions between groups.

3. Results

3.1. Clinical manifestation

Multisystem symptoms, including neuromuscular, liver, kidney, and cardiomyopathy, were observed in 67 patients with respiratory chain complex I deficiency. Neuromuscular dysfunction was an obvious clinical feature. The initial symptom mainly has psychomotor retardation, motor developmental retardation, and weakness. Symptom onset occurred in the first year of life in 41 (61.2%) patients. Intrauterine growth retardation, fetal distress, and gestational diabetes mellitus were observed in the mothers of 11.9% patients. Low birth weight and poor feeding were observed in 7.5% and 6.0% patients, respectively. Nine (13.4%) patients had abnormal family history. Eleven (16.4%) patients had preceding infection or operation history. Onset occurred before the third year of life in 96.9% patients without mtDNA mutation. Onset occurred before the third year of life in 76.5% of patients with mtDNA mutation. There was a significant difference between them ($P = .03$). About 51.5% of patients without mtDNA mutation had weakness, which is higher than 24% patients with mtDNA mutation ($P = .02$). There was no significant difference between patients with mtDNA mutation and patients without mtDNA mutation in other clinical manifestations.

3.2. Activities of mitochondrial respiratory chain complexes

In the 67 patients with complex I deficiency, isolated complex I deficiency was found in 45 (67.2%) patients. The activity of complex I in peripheral blood leukocytes decreased to 11.3 to 28.5 nmol/min/mg mitochondrial protein and 25.7% to 64.8% of the activity in normal controls (44.0 ± 5.4 nmol/min/mg mitochondrial protein). The ratio of the rate of complex I to that of citrate synthase decreased to 24% to 38% (normal controls, 48% ± 11%) (Table 1).

Combined deficiencies of complex I and other complexes were found in 22 (32.8%) patients. Four types of combined deficiency were found, complex I and complex II, complex I and complex IV, complex I and complex V, complex IV and complex V (Table 2). The activity of complex I in these patients decreased to 16.4 to 25.7 nmol/min/mg mitochondrial protein, which was 26% to 48% of the control value. The ratio of the rate of complex I to that of citrate synthase decreased to 20% to 24% (normal controls, 48% ± 11%).

3.3. Analysis of entire mitochondrial gene sequence and core pedigree

mtDNA mutation were identified in 27 (60%) of 45 patients with isolated complex I deficiency and 7 (30.4%) of 22 patients with combined deficiencies of complex I and other complexes (Table 3). The prevalence of mtDNA mutations was significantly higher in patients with isolated complex I deficiency than in patients with combined complex deficiencies ($P = .03$). 3243 A >G, 8993 T >G, 8993 T >C, 10191 T >C, and 10197 G >A were relatively common. Gene analysis was performed in all patient families. Fifteen patients with isolated complex I deficiency and their mothers were found to carry the same mutation. The mutation load of patients ranged from 45.3% to 68.2% and those of their mothers without symptom ranged from 16.5% to 25.2%.

Table 1

Clinical features of patients with respiratory chain complex I deficiency.

Clinical features	Patients with mtDNA mutation		Patients without mtDNA mutation		
	Cases (n=34)	Percentage (%)	Cases (n=33)	Percentage (%)	*P*
Sex (Male/Female)	18/16	50.8	19/14	49.2	
Age at onset					
Neonal period (0–1 mo)	2	5.9	2	6.1	1
Infancy (1 mo–1 y)	15	44.1	22	66.7	.06
Infancy (1–3 y)	9	26.5	8	24.2	.83
Childhood (3–18 y)	8	23.5	1	3	**.03**
Abnormal family history	6	17.6	3	9.1	.48
Abnormal pregnancy	6	17.6	2	6.1	.26
Low birth weight	3	8.8	2	6.1	1
Poor feeding	1	2.9	3	9.1	.36
Preceding infection or operation history	5	14.7	6	18.2	.75
Initial symptom					
Psychomotor retardation	11	32.4	10	30.3	.86
Motor developmental retardation	7	20.6	11	33.3	.24
Motor regression	12	35.3	5	15.2	.05
Weakness, fatigue	8	23.5	17	51.5	**.02**
Epilepsy	10	29.4	12	36.4	.55
Hypotonia	20	58.8	12	36.4	.07
Ataxia	7	20.6	7	21.2	.95
Liver dysfunction	5	14.7	4	12.1	1
Kidney dysfunction	2	5.9	1	3	1
Cardiomyopathy	1	2.9	2	6.1	.61
Brain magnetic resonance imaging (MRI)	16	47.1	13	39.4	
Symmetric lesions of basal ganglia, thalamus	8	23.5	9	27.3	.73
Leukoencephalopathy	6	17.6	2	6.1	.26
Others	2	5.9	2	6.1	1
Normality	18	52.9	20	60.6	.53
Hyperlacticemia	29	85.3	24	72.7	.24
High pyruvate	19	55.9	17	51.5	.72

mtDNA = mitochondrial DNA.
The bold values mean statistical significance.

4. Discussion

Patients with nuclear DNA mutations are more likely to develop early onset in mitochondrial respiratory chain complex I deficiency. Mitochondrial disorders are a cause of neuromuscular diseases. Respiratory chain complex I deficiency is the most frequent mitochondrial disorder presenting in childhood, accounting for up to 30% of cases.[4,5] Clinical manifestation of complex I deficiency is heterogeneous, ranging from severe neurological problems to a near absence of abnormalities. The majority of affected children develop symptoms during the first year of life.[6] The clinical manifestations of Chinese children with complex I deficiency were performed first in our previous study.[7] Swalwell et al[8] reported that the median age at onset was higher in patients with mtDNA (12 months) mutation than in patients with nuclear DNA mutation (3 months). In order to find more characters, more Chinese cases with complex I deficiency were recruited for further research. In this study, psychomotor retardation, regression, and weakness were common, and non-neurological manifestations such as liver diseases, kidney diseases, and cardiomyopathy were also observed, which is consistent with the previous findings. Fifty-two percent of patients without mtDNA mutation had weakness, which is higher than 24% of patients with mtDNA mutation. Symptom onset occurred before the third year of life in 26 patients with mtDNA mutation (76.5%), and in 32 patients without mtDNA mutation (96.9%). Significant difference was observed between them. In the patients with complex I deficiency, the onset may be later in patients with mtDNA mutation than in those without mtDNA mutation.

Table 2

Deficient complexes in the patients with respiratory chain complex I deficiency.

Type	With mtDNA mutations		Without mtDNA mutation		
	cases	%	cases	%	*P*
Isolated complex I deficiency	27	79.4	18	54.5	
Combined complex defects	7	20.6	15	45.5	**0.03**
Complexes I and II	1	2.9	0	0	
Complexes I and IV	2	5.9	7	21.2	
Complexes I and V	4	8.8	8	24.2	

mtDNA = mitochondrial DNA.
The bold values mean statistical significance.

The patients with complex I deficiency of peripheral leukocytes may be more likely to be caused by mtDNA mutation. In most centers, complex I deficiency is diagnosed by spectrophotometric assay in biopsied tissue. The ideal tissue is muscle, liver, or other affected tissues. In patients with a respiratory chain complex deficiency in whom both lymphocytes and skeletal muscle activities were investigated, 50% expressed the deficiency in both tissues, 45% had only a muscular expression, and 5% expressed the deficiency only in lymphocytes.[9] Complex I activity was markedly lower in muscle than in peripheral leukocytes. Only a small number of patients with complex I deficiency presented

Table 3

mtDNA mutations in patients with respiratory chain complex I deficiency.

Gene	Mutation	Translation product	Case number	Frequency	Country and time*
ND1	3697 G >A	G-S	2	2.99	Australia 2004
ND1	3890 G > A	R-Q	2	2.99	Sweden 2008
ND3	10158 T >C	S-P	1	1.49	Italy 2004
ND3	10191 T >C	S-P	4	5.97	UK 2001
ND3	10197 G >A	A-T	3	4.48	China 2009
ND4	11777C >A	R-S	1	1.49	Japan 2003
ND5	12706 T > C	F-L	2	2.99	UK 2002
ND5	13513 G > A	D-N	3	4.48	USA 1997
ND5	13514 A > G	D-G	3	4.48	Italy 2001
ND6	14459 G > A	A-V	1	1.49	USA 1995
ND6	14487 T > C	M-V	1	1.49	Italy 2007
ATP6	8993 T > C	L-P	3	4.48	Netherlands 1993
ATP6	8993 T > G	L-R	3	4.48	England 1990
tRNA-Leu	3243 A > G	$tRNA^{Leu}$	5	7.46	Taiwan 1993

* The mutations have been reported by other countries for the first time.

with the decreased activities in peripheral leukocytes. It was previously believed that complex I deficiency due to mtDNA mutations accounted for only 5% to 10% of pediatric complex I deficiency cases.[10,11] Studies of several cohorts of patients with complex I deficiency have suggested a fairly uniform prevalence of causative mtDNA mutations of 20% to 30%.[8,12] Many studies have reported mutations in nuclear genes encoding subunits, assembly factors, or related proteins in 20% to 25% of patients with complex I deficiency.[13,14] The recent study showed that mtDNA mutation was identified in 27 (40.3%) of 45 patients with isolated complex I deficiency. Patients with complex I deficiency in peripheral leukocytes may be more likely to carry mtDNA mutation in China. 3243 A >G, 8993 T >G, 8993 T >C, 10191 T >C, and 10197 G >A were detected, which was different from our previous findings.[7] Common mtDNA mutation was not identified in China based on these studies.

Usually, clinical heterogeneous of complex I deficiency can be explained by mtDNA mutant load changes. Majamaa et al[15] reported that the median value for the mutant load among probands is 23% in blood and 67% in muscle. Hammans et al[16] reported that the m.3243 A >G mutant load present in blood is significantly greater in symptomatic than asymptomatic subjects and was correlated with a clinical severity score. Elson et al[17] found that the most important factor in predicting whether a mutation can be transmitted to offspring is whether the mutation is selected against in blood. The threshold of transmitted mt-tRNA mutations does not significantly differ between those in the muscle and sporadic mutations. However, the level of sporadic mutations (mean 8%) in blood is significantly lower than that of transmitted mutations (mean 42%).[17] Our previous study showed that 6 mothers transmitted the m.3243A > G mutation to their children. The mutations ranged from 14.1% to 28.6% (mean 20.4%) in blood. This study found that mtDNA mutation of 15 patients with isolated complex I deficiency was from their mothers. The mutation load of patients ranged from 45.3% to 68.2%, and those of their mothers without symptom ranged from 16.5% to 25.2%. The result was consistent with the previous report.[7] These data indicated that mtDNA mutation detected in blood may be more likely to be transmitted to offspring.

Author contributions

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