

## Original Article

# Clinical features of MELAS and its relation with A3243G gene point mutation

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**Abstract:** Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) mostly occur in children. The point mutation A3243G of mitochondrial DNA (mtDNA) may work as a specific bio-marker for mitochondrial disorders. The related clinical features, however, may vary among individuals. This study therefore investigated the relation between MELAS clinical features and point mutation A3243G of mtDNA, in an attempt to provide further evidences for genetic diagnosis of MELAS. Children with MELAS-like syndromes were tested for both blood lactate level and point mutation A3243G of mtDNA. Further family study was performed by mtDNA mutation screening at the same loci for those who had positive gene mutation at A3243G loci. Those who were negative for A3243G point mutation were examined by muscle biopsy and genetic screening. Both clinical and genetic features were analyzed. In all 40 cases with positive A3243G mutation, 36 children fitted clinical diagnosis of MELAS. In other 484 cases with negative mutation, only 8 children were clinically diagnosed with MELAS. Blood lactate levels in both groups were all elevated ( $P>0.05$ ). In a further genetic screening of 28 families, 10 biological mothers and 8 siblings of MELAS children had positive A3243G point mutations but without any clinical symptoms. Certain difference existed in the clinical manifestations between children who were positive and negative for A3243G mutation of mtDNA but without statistical significance. MELAS showed maternal inheritance under most circumstances.

**Keywords:** MELAS, point mutation, mitochondrial DNA, maternal inheritance

## Introduction

As one important sub-type of mitochondrial encephalopathy, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) mostly occur in childhood but without clear illustration of pathogenesis. Common clinical symptoms of MELAS vary from child to child, including coma, dementia, epilepsy, aphasia, vomiting, fever, weakness, headache, ataxia, external ophthalmoplegia, hemiparesis, periodic encephalopathy, audiovisual disorder, hypothyroidism, hirsutism and dwarfism [1, 2]. Due to the lack of specific laboratory indicator, it has been difficult for making a definitive diagnosis of MELAS, although the occurrence of fragile red edged fibers in muscular pathological examination has been postulated [3]. With the advancement of genetic studies, it has been reported that the mutation at loci A3243G of mitochondrial DNA (mtDNA) played an important role in the occurrence of mitochondrial

encephalopathy. Due to the unique cytoplasmic distribution, mtDNA has certain differences regarding its inheritance mechanism compared to nuclear DNA, as various phenomena including higher mutation rates, maternal inheritance, heterozygous, genetic drift may occur more frequently in mtDNA [4]. Novel mutation loci for MELAS have been identified, such as A3252G, T3271C, A3260G, T7512C, G583A, G1642A and T3291C [5]. Epidemiological surveys have contributed more than 80% of MELAS cases into point mutation A3252G, which was the most prevalent point mutation in Chinese Han people with MELAS [6]. When comparing patients with gene mutation at different loci, various clinical symptoms may occur. However, no study has been performed regarding the correlation between such clinical symptoms and genetic mutation. This study utilized PCR-restriction fragment length polymorphism (RFLP) technique to investigate the relationship between all clinical manifestations and mtDNA

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**Table 1.** General information of patients

	Mutation positive	Mutation negative
N	40	484
Sex ratio (M/F)	23/17	257/227
Average age (years)	8.2±4.9	7.6±3.9
Average disease course (years)	2.3±0.8	2.2±1.3

mutation patterns, in order to analyze the genetic feature of the disease.

## Materials and methods

### *Patients and inclusive/exclusive criteria*

We adopted the primary diagnostic criteria of MELAS as previously established [7], including: (1) Brain stroke before 40-years old; (2) Manifested with progressive encephalopathy with dementia and seizure; (3) Multiple systems including central and peripheral organs affected; (4) Hyperlactacidemia; (5) Cerebral infarction/atrophy and basal ganglia affected by head CT; (6) No other encephalopathy or metabolic disorders. In general, individuals who fitted (4), (5) and (6), plus any one of (1), (2) or (3) were diagnosed with MELAS-like cases.

Further confirmed diagnosis was made as previously reported [8]. In brief, a confirmed diagnosis was made based on the primary screening as abovementioned plus: (1) atypical red edged fiber (RRF) in muscle biopsy by succinate dehydrogenase (SDH) staining; (2) Negative for sub-sarcolemma under cytochrome-c (COX) staining; and (3) positive point mutation for A3243G in genetic screening.

Those children who were positive for A3243G point mutation and fitted MELAS clinical symptoms were classified as mutation-positive group. Those who fitted clinical symptoms of MELAS but had negative A3243G mutation and negative for COX or RRF staining were classified in mutation-negative group. This study has been pre-approved by the ethical committee of our hospital and has obtained written consents from all children's guardians.

### *Genetic screening for point mutation*

We adopted PCR-RFLP technique for screening of A3243G point mutation of mtDNA. In brief, peripheral blood samples were collected from patients and extracted for genomic DNA. The

PCR utilized specific primers for A3243G mutation (Forward, 5'-CCTCC CTGTA CGAAA GGACA-3'; Reverse, 5'-CACCC TGATC AGAGG ATTGA G-3') under the following condition: 95°C pre-denature for 5 min, followed by 30 cycles each containing 94°C denature for 30 sec, 60°C annealing for 30 sec and 72°C elongation for 60 sec. The reaction ended after a further 72°C elongation for 7 min. PCR products were then cut by Apal restriction enzyme, which can cut the mutated gene into two fragments (130 and 423 bp) while leaving the wild type gene intact (553 bp). Digestion fragments were finally separated under agarose gel electrophoresis. The gel image was captured and analyzed by Quantity One software (BioRad, US).

### *Statistical analysis*

All collected data were analyzed by SPSS 21.0 software package. The correlation between MELAS clinical symptoms and A3243G point mutation was processed by one-factor correlation analysis. A statistical significance was defined when  $P < 0.05$ .

## Results

### *General information of patients*

As shown in **Table 1**, among all 524 cases with MELAS-like symptoms, 40 of them were positive for mutation while 484 cases were negative for the point mutation. General information including onset age, disease course and sex ratio had no significant difference between those two groups ( $P > 0.05$ ).

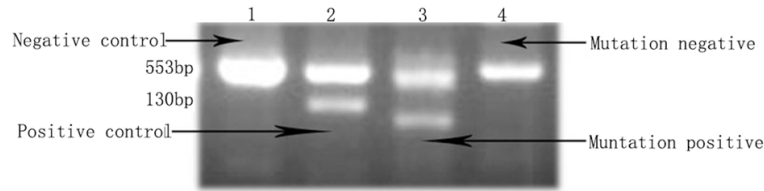
### *Blood lactate level assay*

Serum assays showed that the blood lactic acid level was between 4.2 and 9.9 mM in mutation positive group, and was between 2.3 and 10.8 mM in mutation negative group. Both groups showed higher lactate level than normal people (0.5 to 2.0 mM) but without any statistically significant difference ( $P > 0.05$ ).

### *Gene screening for A3243G mutation*

**Figure 1** showed the RFLP pattern of both mutation positive (Lane 3) and mutation negative (Lane 4) individuals. Mutant gene form had two restriction bands of 423 and 130 bp while wild type form had one single band at 553 bp after restriction digestion.

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**Figure 1.** RFLP patterns of A3243G mutation. Lane 1, negative control; Lane 2, positive control; Lane 3, mutation negative; Lane 4, mutation positive.

**Table 2.** Clinical features of MELAS

Symptom	Mutation positive (N=36)	Mutation negative (N=8)	$\chi^2$	P
Weakness	30	5	0.215	0.764
Dementia	28	7	0.042	1.000
Seizure	35	6	0.194	0.773
Omitting	17	0	3.557	0.092
Fever	16	0	3.357	0.095
Hirsutism	30	0	6.116	0.018
Dwarfism	35	1	4.706	0.037
Vision loss	22	5	0.001	1.000
Fever	7	2	0.080	1.000
Vomiting	5	1	0.008	1.000
Aphasia	8	2	0.018	1.000
Coma	9	3	0.278	0.686
Cerebral stroke	11	3	0.073	1.000
Headache	4	0	0.873	1.000
Auditory disorder	3	0	0.657	1.000

### Clinical features and its relation with A3243G point mutation

In mutation positive group, patients were mainly manifested as dwarfism and hirsutism, along with other features including weakness, seizure, fever, aphasia, omitting, progressive dementia and vision loss. In some patients, there were also headache, coma, cerebral stroke and auditory dysfunctions. A total of 26 cases were complicated with epilepsy. In mutation negative group, patients also showed seizure, weakness, dementia and vision loss but no hirsutism or dwarfism (but not in one case). A tabulated description of all major clinical features was shown in **Table 2**. Some of those features showed significant difference between two groups ( $P < 0.05$ ).

### Family survey of MELAS patients

In mutation positive group, four children had definitive family history, as two cousins of

patients had been diagnosed with MELAS by muscular biopsy when alive. One patient's sister has been misdiagnosed with renal tubular acidosis since two years old, and manifested with progressive dementia and ptosis at 10 years old. The patient's mother showed general weakness and lower body weight but without A3243G mutation. In mutation negative group, one child's mother had psychiatric disorders. One sibling of this patient also died without definitive reason. No family history has been discovered in any patient in the mutation negative group.

### Family screening of A3243G mutation

We also performed the genetic screening targeting A3243G mutation in a total of 74 family members in 28 families with mutation positive MELAS child. A total of 10 biological mothers were positive for A3243G mutation, along with

8 siblings. All these carriers had no clinical symptoms. Other family members had no genetic mutation at the given locus.

### Discussion

Mitochondrial encephalopathy was a multi-system heterogeneous disease that has both genetic and acquired factors. As the organelle for energy transformation inside eukaryotic cells, mitochondria is unique with other cellular compartments as it has its self-organized DNA and unique genetic mechanism. With the advancement of genetic diagnosis, the mutation of mtDNA has been recognized to be related with various mitochondria diseases including MELAS [9, 10]. Studies have revealed the A3243G mutation as the most commonly occurred pathogenic mutation locus of this disease. The location of this mutation is within UUR coding region of tRNA. The A to G substitution lead to per-termination of transcription, impeding expression of normal rRNA, thus

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compromising mitochondrial protein synthesis, ATP production and body metabolism [11, 12]. MELAS may occur in all age groups but predominantly during childhood with various clinical manifestations including periodic headache, seizure, vomiting, vision loss and weakness. Currently, diagnosis is mainly dependent on the occurrence of ragged red fiber and negative cytochrome C staining in muscular biopsy. Atypical features including various central nervous injury and progressive extraocular paralysis but without cerebral stroke may also occur in children who also had growth retard, seizure and cognitive disorder but perhaps no ragged red fiber in biopsy [13-15]. This study observed the correlation between A3243G mutation in MELAS children and their clinical features, thus providing references for genetic diagnosis.

In clinical practice, similar manifestations exist between MELAS patients who were positive for mtDNA point mutation and for those who were negative for point mutation, further complicating the sub-typing of patients. As negative results in pathological examination may not completely indicate the clinical sub-type, further genetic screening can work as one laboratory index. The correlation between mutation and clinical features, however, remained poor understood [16-18]. This study performed a correlation analysis between the clinical features and MELAS patients who were positive or negative for mtDNA point mutation, by the means of one-factor or multi-factor correlation analysis. Our results showed mutation positive patients had disease-related features including weakness, dementia, seizure, vomiting, fever, hirsutism and dwarfism, while mutation negative patients had other disease-related manifestations including weakness, seizure, dementia and vision loss. By a multi-factor regression analysis, the most commonly occurred clinical symptoms in mutation positive ones were (in descending order) weakness, hirsutism, dementia and seizure; whilst mutation negative patients most frequently had seizure, dementia, vision loss and weakness.

Meanwhile we also performed family survey on families of MELAS patients, including mutation positive and negative ones. In a genetic screening for mtDNA A3243G mutation, we found 10 mothers and 8 siblings of mutation positive children also carried such point mutations. In mutation negative group, however, no A3243G

genotype has been detected. These results support the maternal inheritance pattern of A3243G mutation, consistent with previous reports [19, 20].

In summary, certain but not significant differences exist in clinical features between MELAS patients with positive and negative A323G mutation, which follow a maternal inheritance pattern. Our study for the first time provides correlation between clinical features and mtDNA mutation of MELAS, although further independent multi-centered survey is required for the substantiation.

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### Disclosure of conflict of interest

None.

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