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Case Report

A Patient with MELAS Syndrome Carried an M.3243A>G Mutation in Mitochondrial DNA and Multiple Nuclear Genetic Variants: A Case Report

Xin Chu [1]***, Jie Dai*** [2]***, Hengxiang Cui*** [3]***, Hailin Shen*** [2]***, Qi Wu*** [1]***, Xiangyang Zhu*** [2]***, *Baofeng Zhu*** [1]

1. *Emergency Department, Second Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, China*
2. *Neurology Department, Second Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, China*
3. *Clinical Medical Research Center, Second Affiliated Hospital of Nantong University, Nantong, Jiangsu Province, China*

***Corresponding Author:** Email: Baofengdr123@outlook.com

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Abstract

We discuss the involvement of nuclear genetic variants correlating to observed phenotype in this case study. In January 2020, the 19-year-old boy from Nantong, Jiangsu Province, China with epilepsy symptom was identified to have myelin loss in the motor and sensory nerves in the electromyogram examination. Brain magnetic resonance imaging (MRI) demonstrated high-intensity areas of small multifocal gray matter regions in the bilateral temporal, parietal, and occipital lobes. In the serum of the patient, the levels of lactate dehydrogenase (LDH) and lactic acid were higher than the normal range values in multiple tests. By subsequent whole exome sequencing (WES) including analysis of the mitochondrial genome, the patient was revealed to carry an m.3243A>G mutation in mitochondria *MTTL1* gene which was confirmed by direct Sanger sequencing analysis. Thus, disease of the patient was diagnosed as mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. According to WES analysis, the patient also carried multiple homozygous variants, which correlating to myelinloss and epilepsy in nuclear genes. The peripheral neuropathy of the patient carrying single mitochondrial m.3243A>G mutation could be caused by multiple nuclear DNA defect.

Keywords: Mitochondrial DNA mutation; *MELAS* syndrome; Epilepsy; Myelinloss; Case report

Introduction

Being the most common mitochondrial DNA (mtDNA) mutation, m.3243A>G in the mtDNA-encoded tRNA leucine 1 (*MTTL1*) gene affects proper incorporation of the amino acid leucine in mtDNA encoded proteins. It causes a multiple clinical manifestations, of which the most common ones are mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome or maternally inherited diabetes and deafness (MIDD) syndrome (with 10% and 38% of m.3243A>G carriers, respectively) (1). The MELAS syndrome is a mitochondrial disorder resulting in anatomohistopathological and clinical findings with progressively neurodegenerative and eventually life-threatening that is caused by the decreased ability

of cells to produce sufficient energy in form of adenosine 5'-triphosphate (2).

The m.3243A>G mutation in MT-TL1 is the most common mitochondrial DNA mutation that led to a wide spectrum of disorders in a maternally inherited pedigree. Not all the individuals would be affected for carrying the m.3243A>G mutation. In mothers carrying the m.3243A>G mutation with a heteroplasmy level of below 25%, 30% of the offsprings were unaffected (3). The patients with the m.3243A>G mutation typically had intact myelin; however, some of them exhibited myelin pallor and selective myelin loss (4). In this emergency treatment case, the young patient having epilepsy symptom was revealed to have the myelin loss inferred from the decrease of nerve conduction velocity of right common peroneal nerve and the elicitation failure of the compound muscle action potential (CMAP) of right ulnar and bilateral tibial motor nerves, the sensory nerve action potential (SNAP) of bilateral median and bilateral sural. Further we identified the patient carried the m.3243A>G mutation accompanied with risk nuclear genetic mutations related to myelin loss and epilepsy by whole exome sequencing (WES) including analysis of the mitochondrial genome.

Case presentation

The study approved by the Ethics Committee of Nantong University Affiliated Second Hospital (No. 2020-0306) was conducted in accordance with the Declaration of Helsinki. Consent was obtained from all the individuals involved in the present study, including the family members, and participants agreed to provide blood samples.

The patient, who was a 19-year-old boy having fever and cough symptoms 10 days before the attack, was send to our hospital for treatment in January 2020 because of paroxysmal tetany in a state of dottiness for 12 hours. For rescue he was performed with the tracheal cannula after the patient received propofol-mediated sedation. Thereafter he reemerged the paroxysmal tetany in a state of dottiness, hearing loss and tinnitus symptoms. Then the patient's tendon reflex of the extremities was disappeared. And his muscle tension was reduced and his muscles of intercostal muscles and extremities were obviously atrophied. The patient once got visual hallucinations before being given antiepilepsy and nerve nutrition treatment. During the course of the disease, the patient recurred limb convulsions, visual hallucinations, hearing loss, headache, dizziness and discomfort, accompanied by repeated pneumothorax and no obvious changes in muscle strength. With a history of "myocarditis", he had a thin body and mediocre school record. His mother had congenital strabismus, and his uncle-in-law, a son and a daughter of his uncle-in-law had epilepsy. During the course of the disease, the patient recurred limb convulsions, visual hallucinations, hearing loss, headache, dizziness and discomfort, accompanied by repeated pneumothorax.

On hospital admission, blood tests for this patient for assessment of liver and kidney function and electrolyte level analysis were conducted. The result showed that the levels of serum lactate dehydrogenase (LDH) and Lactic acid were higher than the normal range values (Tables 1 and 2).

Table 1: The levels of lactic acid and glucose in serum of the patient on indicated day

Item	*Feb18 Values*	*Feb25 Values*	*Mar28 Values*	*Mar4 Values*	*Mar13 Values*	*Reference Range*
Lactic acid	4.32*	4.30*	3.95*	2.83*	2.74*	1.19-2.09 mmol/L
Glucose	5.05	7.79*	5.97	6.01	7.15*	3.3-6.1 mmol/L

*, higher than the reference range; the tests were conducted on indicate date.

While the levels of creatinine, uric acid, sodium ion and chloride ion were lower than the normal range values (Table 2). During diagnosis, the ganglioside antibodies in the patient's serum and cerebrospinal fluid (CSF) were also evaluated. The serum of the patient contained the IgM antibodies against ganglioside GM4 and ganglioside GQ1b/GD3 but no ganglioside-monosialic acid (GM1) antibodies. For the results from ganglioside antibodies, the disease of the patient once suspected as Guillain–Barré syndrome.

Table 2: The indices for estimating renal function and electrolyte balance in serum

Item	*Feb19 Values*	*Feb28 Values*	*Mar14 Values*	*Mar8 Values*	*Mar22 Values*	*Mar27 Values*	*Reference Range*
Lactate-dehydrogenase	583*	347*	205	170	262*	254*	80-245 U/L
Total bilirubin	2.9	5.2	2.4	2.3	1.1●	1.5●	1.7-17.1μmol/L
Direct bilirubin	1.0	1.1	1.1	0.8	0.4	1.5	0-7 μmol/L
Indirect bilirubin	1.9	4.1	1.3	1.5	0.7	0.3	3-16 μmol/L
Creatinine	14.0●	34.0	16.0●	14.0●	16.0●	15.0●	30-120 μmol/L
Uric acid	126.0●	54.0●	94.0●	82.0●	100.0●	79.0●	208-428 μmol/L
Sodium ion	138.7	133.6●	136.0●	143.9	139.2	134.3●	137-147 mmol/L
Chloride ion	103.6	97.1●	95.8●	100.1	97.6●	92.2●	99-110 mmol/L
Calcium ion	2.28	2.52*	2.56*	2.51*	2.49	2.35	1.96-2.5 mmol/L
Magnesium ion	0.72	0.61●	1.05	0.81	0.84	1.12	0.7-1.2 mmol/L
Hydrogen-carbonate	17.5●	16.6●	26.9	28.2	23.2	26.7	22-29 mmol/L

*, higher than the reference range; ●, lower than the reference range; the tests were conducted on indicate date

In brain magnetic resonance imaging (MRI), DWI showed a linear of bilateral and symmetrical signal in high intensity at the level of temporo-parietal-occipital cortex with reduced ADC map values (Fig. 1A, B).

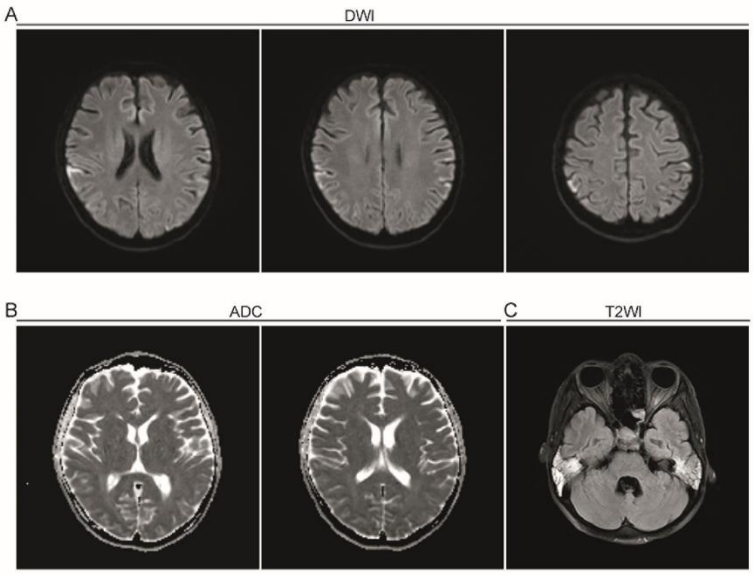

Fig. 1: Sequential changes in MRI. (A, B) DWI showed a linear of bilateral and symmetrical signal in high intensity at the level of temporo-parietal-occipital cortex (A) with reduced ADC map values (B). (C) T2WI image demonstrated quasi circle high intensity signal in the left ethmoidal sinus

T2WI image demonstrated quasi circle high intensity signal in the left ethmoidal sinus (Fig. 1C). Parameters recorded from motor nerves included the compound muscle action potential (CMAP), motor amplitude in millivolts (mV), the distal motor latency (DML) in milliseconds (ms), conduction velocity in meters per second (m/s), measured from baseline to the negative peak of the CMAP waveform, the time from the stimulus to the onset of motor activity, and shortest F-wave latency (FWL) in ms (Table 3). Sensory nerve measures included the sensory nerve action potential (SNAP), sensory amplitude in microvolts (μV), and the conduction velocity in meters per second (m/s) (Table 4).

Table 3: Electrophysiological studies of the motor nerve function in the patient

Segment	*Onset Latency (ms)*	*Norm Onset Latency (ms)*	*Amplitude (mv)*	*Normal Amplitude (mv)*	*Velocity*	*Normal Velocity (m/s)*
Left Ulnar						
ADM-Wrist	2.3	<3.3	1.2	>6.0	-	
Wrist-Elbow	6.5		1.0		52.4	>49
Elbow-Armpit	11.2		1.0		60.6	>49
Right Ulnar						
ADM-Wrist	2.5	<3.3	1.0	>6.0	-	
Wrist-Elbow	6.2		1.0		54.1	>49
Elbow-Armpit	11.8		1.0		52.7	>49
Left Median						
Wrist-APB	3.0	<4.4	2.6	>4.0	-	
Wrist-Elbow	7.3		2.1		57.0	>49
Elbow-Armpit	11.4		2.1		61.0	>49
Right Median						
Wrist-APB	2.9	<4.4	2.6	>4.0	-	
Wrist-Elbow	7.8		2.2		53.1	>49
Elbow-Armpit	11.8		2.3		60.0	>49
Left Tibial						
Ankle-AH	5.8	<6.3	0.2	>4.0	E.F.	>41
Popliteal fossa-Ankle	14.2	<5.8	0.2	>3.0	45.2	>41
Right Tibial						
Ankle-AH		<6.3		>4.0	E.F.	>41
Left Common Peroneal						
EDB-ankle		<6.5		>2.0	E.F.	>44
Right Common Peroneal						
EDB-ankle	4.1	<6.5	0.6	>2.0	-	>44
Down knee-ankle	12.4	<6.6	0.5	>3.0	41.0	>44
Up-down knee	14.8	<6.6	0.5	>3.0	45.8	>44

ADM, abductor digiti minimi; APB, adductor pollicis brevis; AH, abductor hallucis; EDB, extensor digitorum brevis; -, no testing; E.F., Elicitation Failure

Table 4: Electrophysiological studies of the sensory nerve function in the patient

Segment	*Onset Latency (ms)*	*Norm Onset Latency (ms)*	*Amplitude (μv)*	*Normal Amplitude (μv)*	*Velocity*	*Normal Velocity (m/s)*
Left Ulnar ADM-Wrist	2.8	<3.1	4.1	>17	60.0	>50
Right Ulnar ADM-Wrist	3.1	<3.1	1.5	>17	54.2	>50
Left Median Wrist-APB		<3.5		>20	E.F.	>50
Right Median Wrist-APB		<3.5		>20	E.F.	>50
Left Sural Shank -Lateral malleolus		<4.4		>6.0	E.F.	>40
Right Sural Shank -Lateral malleolus		<4.4		>6.0	E.F.	>40

ADM, abductor digiti minimi; APB, adductor pollicis brevis; E.F., Elicitation Failure

In the electromyogram examination, the patient had injury of the extremity in peripheral nerve including both the motor and sensor nerves. There were great decreases in the CMAP motor amplitude in nerves of bilateral median, bilateral ulnar, left tibial and right common peroneal, which indicated that these motor nerves carried axonal damage (Table 3). And the nerve conduction velocity (NCV) of right common peroneal nerve was decreased (Table 3). The left common peroneal CMAP and F-wave of right ulnar and bilateral tibial motor nerves were failed to be elicitated. Meawhile the SNAP of the bilateral median and bilateral sural were failed to be elicitated (Table 4). These results indicated that the motor and sensory nerves could have acute myelin loss.

Genomic analysis

The whole nuclear gene exomes and mitochondrial genome of peripheral blood or saliva DNA were sequenced from the probands using xGen Exome Research Panel v1.0（Integrated DNA Technologies, Inc.，USA）and HiSeq X10 Sequencing System (Illumina Inc., San Diego, CA, USA). Gene variants were searched using a review of the scientific and clinical literature (Supplement data). In this case the peripheral neuropathy and epilepsy of the patient carrying single mitochondrial m.3243A>G mutation could be caused by multiple nuclear DNA defect including gene variations in Table 5.

Table 5: The homozygous nuclear genetic variants correlating to myelinloss and epilepsy in WES analysis

Gene	*Chr: position*	*Ntb*	*AA*	*dbSNP137*	*Related phenotype/ disease*	*Ref*
NRG1	chr8:32453358	c.113G>A	R38Q	rs3924999	Myelin loss	Ref12-15
RPV1	chr17:3486702	c.1406C>T	T469I	rs224534	MS	Ref16
ABCG8	chr2:44071743	c.161A>G	Y54C	rs4148211	MS	Ref17
HSPA1L	chr:31778272	c.1478C>T	T493M	rs2227956	MS	Ref18
IGHMBP2	chr11:68703959	c.2011A>G	T671A	rs622082	CMT*	Ref19
SCN1A	chr2:166892788	c.3199G>A	A1067T	rs2298771	Epilepsy	Ref21

■Ntb, DNA mutation sites in indicated genes; AA, mutation sites in amino acid of indicated genes; dbSNP137, All SNPs were identified using the NCBI dbSNP137; Ref, The reference related to the variation

For some cases of MELAS syndrome appear to occur as the result of a new spontaneous mutation in a mitochondrial gene and are not inherited. Thus we use Sanger sequence to analyze the MTTL1 gene of his family. Sanger sequencing was performed on DNA from probands for genotype confirmation. The m.3243A>G mutation of the patient could be inherited from his mother according to the Sanger sequencing analysis (Fig. 2).

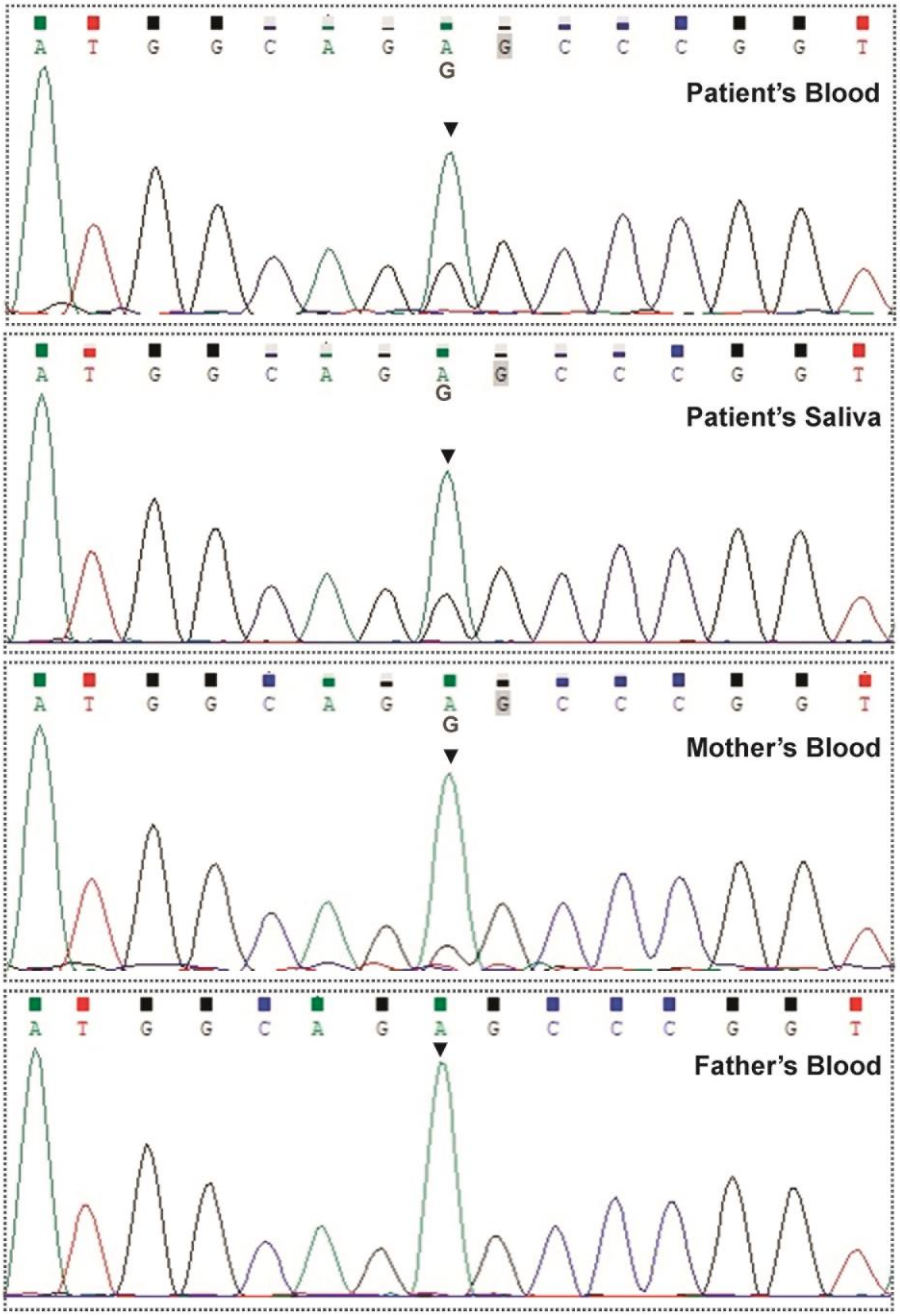

Fig. 2: Direct sequencing of MTTL1 from blood or saliva of the patient's family revealed the m.3243A>G mutation

Discussion

In this case study, the patient was diagnosed with epilepsy, being identified to have axonal damage, myelin loss and a linear of bilateral and symmetrical signal in high intensity at the level of tempo-ro-parietal-occipital cortex with reduced ADC map values. In the serum, there were ganglioside GM4 IgM and ganglioside GQ1b/GD3 IgM antibodies. For both the two antibodies are associated with Guillain–Barré syndrome, the disease of the patient was once supposed to be Guillain–Barré syndrome (5, 6). By the WES including analysis of the mitochondrial genome, the patient was revealed to carry an m.3243A>G mutation in mitochondria *MTTL1* gene. Combining the phenotypes including epilepsy, myelin loss and high level of lactic acidosis in serum, the disease was diagnosed as MELAS (7). We also analyzed the result from mitochondrial genome, because only about 80% of MELAS syndrome carry an m.3243A>G mutation in the mitochondrial *MTTL1* gene (7), and it has been revealed that mutations in MT-TQ, MT-TH, MT-TK, MT-TS1, MT-ND1, MT-ND5, MT-ND6, and MT-TS2 in mtDNA were also associated with MELAS syndrome. We found there was an m.356A>G mutation in MT-ND6 (Supplement data), but there was lack of evidence supporting that the mutation participated the MELAS related symptoms. Thus we incline to the MELAS syndrome was caused by m.3243A>G mutation in mitochondria *MTTL1*.

For some cases of MELAS syndrome appear to occur as the result of a new spontaneous mutation in a mitochondrial gene and are not inherited. Thus we use Sanger sequence to analyze the *MTTL1* gene of his family. The result showed that the m.3243A>G mutation of the patient could be inherited from his mother according to the Sanger sequencing analysis (Fig. 2). In a previous study, 7 of 32 patients with m.3243A>G mutation fulfilled the electrodiagnostic criteria for polyneuropathy, disclosing a peripheral neuropathy with mixed axonal and demyelinating features in six cases and uniform demyelinating features in one case (8). In this study, the patient also showed a peripheral neuropathy with mixed axonal and demyelinating features according to the electromyogram examination.

Though we know that MELAS patients had multiplex phenotypes including sensorineural hearing loss, diabetes and proximal myopathy (9), few study further afforded each phenotype relating genetic risk variations. We further comprehensively analyzed the 495 homozygote variations in the WES analysis (Supplement data). The result showed that the patient carried rs3924999 vitiation carrying R38Q mutation in neuregulin 1 gene (NRG1). Axonal neuregulin-1 regulates myelin sheath thickness (10) and myelin sheath thickness is a quantifiable readout for Nrg1 activity (11). The rs3924999 is one of the six core SNPs within the NRG1 gene identified as promising schizophrenia risk gene (12). In addition, the missense mutation on rs3924999 of the NRG1 may have a functional effect on prepulse inhibition in both healthy control and schizophrenia populations (13). This indicated that R38Q mutation in NRG1 could change its function and signaling regulation leading to affecting the myelin sheath thickness and remyelination.

In this case, the patient had multiple homozygote variations related to the risk of multiple sclerosis (MS). These variations included TRPV1: p.T469I(rs224534), ABCG8: p.Y54C (rs4148211), and HSPA1L:p. T493M (rs2227956) (Table 5). MS is an inflammatory disease characterized by myelin loss and neuronal dysfunction. In addition, the patient also carried the IGHMBP2:p.T671A(rs622082) variation correlating to Charcot-Marie-Tooth disease (CMT) (14) which is characterized to have the myelin loss phenotype. Thus these variations may contribute to the myelin loss phenotype is this case.

In a cohort study of epilepsy in adults with mitochondrial disease, a prevalence of 34.9% in the most common m.3243A>G mutation was revealed (15). We also revealed the variation related to epilepsy phenotype. The patient had a single polymorphic nsSNP (rs891398) encoding a T125A missense variant in CHRNA2, a known gene for a nocturnal frontal lobe epilepsy syn-

drome (supplement data file- not showed) (16). But it needs further study about whether the rs891398 be associated to the epilepsy phenotype.

Conclusion

This study would encourage us combine the WES method including sequencing of the mitochondrial genome even CNV analysis to help accurate diagnosis.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors. Participants underwent comprehensive physical examinations. Written informed consent was obtained from each participant.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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