

# Whole-exome sequencing reveals a novel mutation of *MT-ND5* gene in a mitochondrial cardiomyopathy pedigree: Patients who show biventricular hypertrophy, hyperlactacidemia, pulmonary hypertension, and decreased exercise tolerance

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## ABSTRACT

**Objective:** The aim of the present study was to determine whether pathogenic mutations were present in families with mitochondrial cardiomyopathy that presented during adolescence.

**Methods:** The proband was a 21-year-old man who presented clinically with palpitations, chest tightness, pulmonary hypertension, and limited exercise tolerance. Cardiac magnetic resonance imaging studies showed biventricular cardiac hypertrophy. We determine whether pathogenic mutations were present by whole-exome sequencing (WES) in families.

**Results:** Screening of the family using tandem mass spectrometry showed elevated lactic acid levels, glutaric aciduria, a mildly increased glutaryl carnitine-to-octanoyl carnitine ratio, and normal blood  $\alpha$ -glucosidase, which was consistent with a respiratory chain complex 1 metabolic disorder. We identified a novel mutation of *MT-ND5*, c.1315A>G (p.Thr439Ala). Skeletal muscle biopsy histology showed predominantly ragged red fibers and few ragged blue fibers, which was consistent with mitochondrial myopathy.

**Conclusion:** In the present study, we identified a novel mutation of *MT-ND5*, c.1315A>G (p.Thr439Ala), in a family pedigree using WES. (*Anatol J Cardiol* 2019; 21: 18-24)

**Keywords:** *MT-ND5*, whole-exome sequencing, cardiomyopathy, pulmonary hypertension

## Introduction

Mutations in mitochondrial DNA protein-coding genes have been shown to be involved in important causes of childhood mitochondrial encephalomyopathies (1). Of the protein-coding genes, mitochondrially encoded nicotinamide adenine dinucleotide hydrate (NADH) ubiquinone oxidoreductase core subunit 5 gene encodes one of the seven subunits comprising complex I of the electron transport chain. *MT-ND5* is recognized as an important gene in the study of the pathogenesis of mutations that affect complex I and result in mitochondrial myopathies, including cardiomyopathies (2). The phenotypes associated with mutations in the gene are often severe and are clinically associated

with a wide range of clinical phenotypes, varying by the age of onset, degree of organ involvement, and degree of myopathy, and include Leigh syndrome and mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) (3, 4). The clinical, imaging, and genetic heterogeneity of pediatric mitochondrial disorders often poses a diagnostic challenge (5, 6).

Genome DNA-encoding sequence enriched by exon trapping technology and analysis by whole-exome sequencing (WES), providing explicit sequence information, greatly promoted the diagnosis of genetic diseases. In the present study, WES was used to screen the disease-causing gene mutation of a family with mitochondrial cardiomyopathy, and the pathogenic gene *MT-ND5* and a novel mutation c.1315A>G (p.Thr439Ala) were detected.

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## Methods

### Ethical approval and patient consent

The Ethics Committee of Zhongshan Hospital, affiliated to Fudan University (no. 2016-16B) approved the study. Consent was obtained from all the individuals involved in the present study, including the family members, and participants agreed to provide blood samples.

### Genetic studies of family members

Owing to the possibility of familial cardiomyopathy, the family underwent genetic studies (Fig. 1a). Detailed clinical data for all members of the family were collected, including physical examination, details of the age of onset of any clinical symptoms, presenting symptoms, serum creatine kinase (CK) levels, cardiac function testing, electrocardiography, and echocardiography and 24-hour Holter monitoring studies, if required (Table 1).

### WES and analyses

The whole exomes of peripheral blood DNA were sequenced from the family members (parent-child trios) using a 39 Mb charge-coupled device capture probe and HiSeq 2500 Sequencing System (Illumina Inc., San Diego, CA, USA) in PE125 formats. The mean coverage of the captured regions was 88× per sample, with 97%–98% exons covered with at least 10× coverage, and an average of 92% vbase call quality of Q30 or greater. Raw sequence reads were mapped against human genome *GRCh37* and analyzed using an in-house method that automatically performed Burrows-

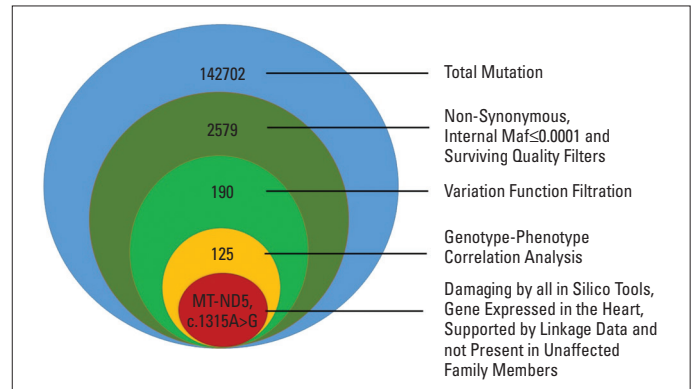


Figure 2. Whole-exome sequencing variants filtering scheme

Wheeler Aligner mapping and FreeBayes Variant Calling Protocol, followed by filtering against in-house and public databases and genetic models (autosomal dominant) fitting (7). Additional automatic filtering was applied to eliminate sequencing artifacts. Gene variants, together with clinical diagnosis keywords, were searched using a review of the scientific and clinical literature (Fig. 2).

### Sanger sequencing and analyses

Sanger sequencing was performed on DNA from probands for genotype confirmation and for the family members for familial co-segregation analysis.

## Results

### Clinical presentation and investigation of the proband

The proband was a 21-year-old man who had limited physical activity since childhood. The patient had an upper respiratory tract infection 1 month prior to hospital admission, followed by palpitations, chest tightness, lower extremity weakness, edema of the lower extremities, fever, cough, expectoration, chest pain, and joint pain. On hospital admission, blood test findings included troponin 0.51 ng/mL, lactic acid 17.0 mmol/L, white blood cell (WBC) count  $15.7 \times 10^9/L$ , C-reactive protein (CRP) 27.56 mg/mL, troponin 1.41 ng/mL, myoglobin 387.9 ng/mL, lactate dehydrogenase (LDH) 1260 U/L, and brain natriuretic peptide (BNP) 1173 pg/mL. Blood gas analysis studies showed a  $CO_2$  partial pressure of 24.3 mm Hg, with an  $O_2$  partial pressure of 112.0 mm Hg. Following the administration of antibiotics, improving circulation and myocardial function, the patient's temperature returned to normal, and the symptoms of chest tightness decreased slightly. The patient was then discharged from the hospital after administering treatment. Following hospital discharge, the patient still had limb weakness with increased chest tightness that required emergency hospital admission.

Clinical laboratory findings at this time included plasma pH 7.39, WBC count  $9.54 \times 10^9/L$ , CRP 12.3 mg/L, CK 188 U/L, CK-MB 62 U/L, CK-MM 126 U/L, cardiac troponin T 0.061 ng/mL, N-terminal pro-B-type natriuretic peptide 877.7 pg/mL,  $CO_2$  pressure 54.30 mm Hg,  $O_2$  pressure 264.0 mm Hg, hemoglobin 12.0 g/dL, hema-

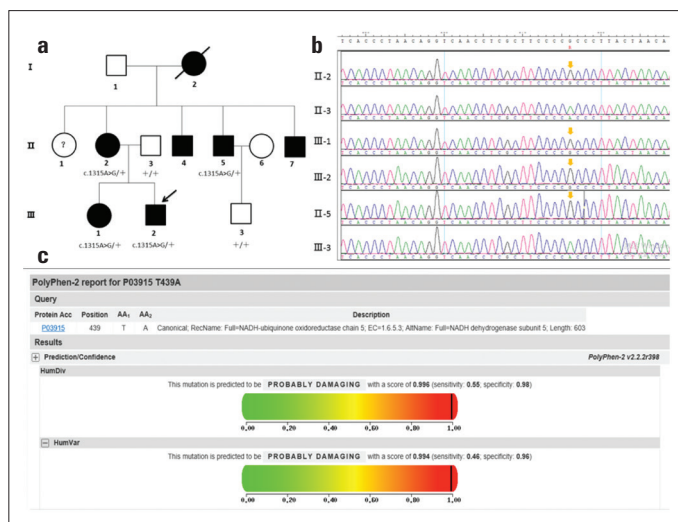
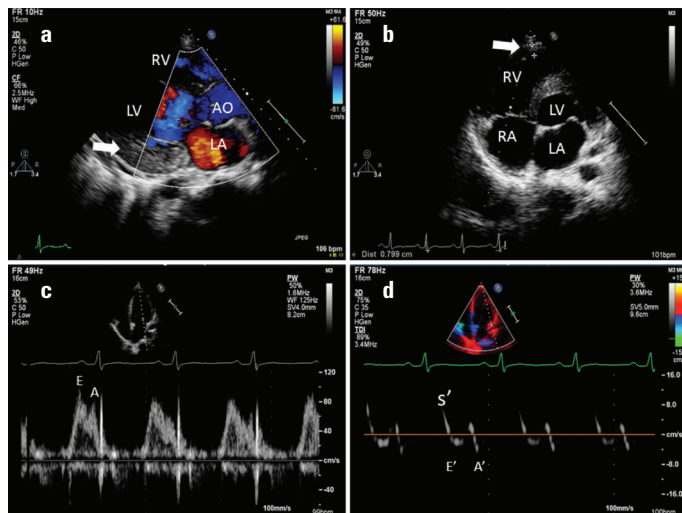


Figure 1. The family pedigree and sequence chromatograms with results of the polymorphism phenotype analysis. (a) The family pedigree studied. Squares represent male individuals, circles represent female individuals, slashes represent deceased individuals, black area is the index patient, and arrow represents the proband. (b) Sequence chromatograms show the presence of mutation *MT-ND5* (c.1315A>G and p.Thr439Ala) in the studied patient (arrow) and its absence in the family. (c) Results of the Polymorphism Phenotyping v2 analysis predicting the physical abnormalities associated with the p.439T>A substitution in the ND5 protein

**Table 1. Clinical presentation of the family members in the present study**

Patient	Sex	Age (years)		ECHO				LVEF (%)	BP (mm Hg)	ECG		
		At testing	At onset	MLVWT (mm)	IVS (mm)	LVPWT (mm)	IVS/LVPWT			Rhythm	Abnormal Q wave	ST-T change
II-2	F	46	29	10	10	9	1.11	61	102/74	Sinus	+	+
II-3	M	50	—	11	11	10	1.10	67	124/70	Sinus	—	+
III-1	F	23	8	10	10	8	1.25	62	114/62	Sinus	—	—
III-2	M	21	5	18	14	18	0.78	55	106/66	Sinus	+	+
II-5	M	52	17	12	12	11	1.09	65	118/76	Sinus	—	+
III-3	M	28	—	11	11	11	1.00	66	132/68	Sinus	—	—

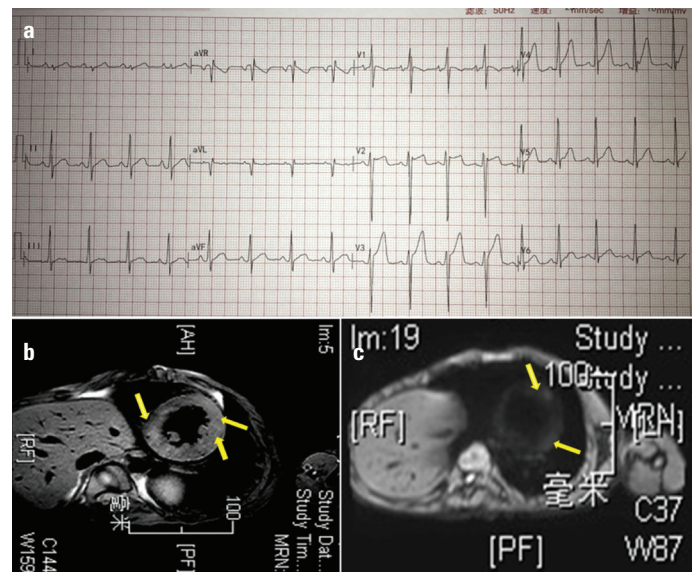
ECHO - echocardiography; MLVWT - maximum left ventricular wall thickness; IVS - interventricular septum; LVPWT - left ventricular posterior wall thickness; LVEF - left ventricular ejection fraction; BP - blood pressure; ECG - electrocardiograph



**Figure 3.** Two-dimensional echocardiography of the proband. (a) The left ventricular chamber size of the proband is normal, but the left ventricular wall is thickened, measuring between 13 and 18 mm (arrow). (b) There is no abnormality of the left ventricular systolic activity. The left ventricular ejection fraction is 57%. The right ventricular free wall has a thickness of 7 mm (arrow). (c, d) The right ventricular chamber size is normal. Doppler echocardiography shows an impaired left ventricular diastolic function. Pulsed Doppler imaging ( $E/A > 1$ ); Doppler tissue imaging ( $E'/A' < 1$ ) (arrow)

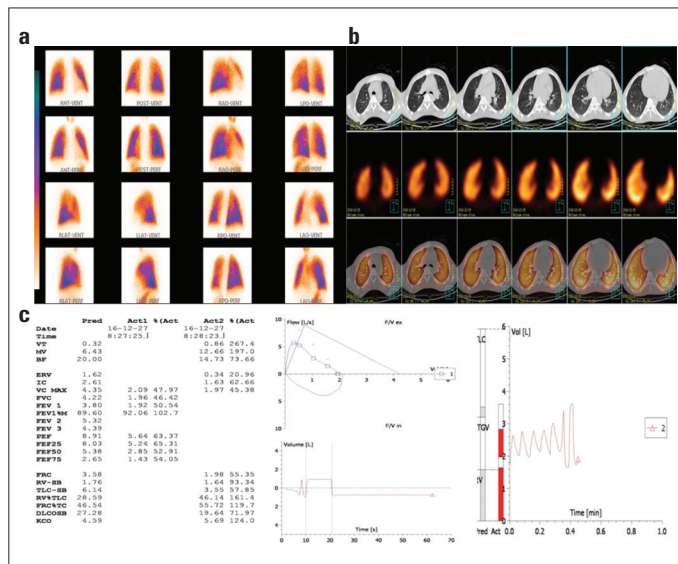
tocrit 36.90%, and  $O_2$  saturation 100.6%. An electrocardiogram (ECG) showed sinus tachycardia of 102 beats per minute. Echocardiography showed thickening of the left and right ventricular wall, the left ventricular diastolic dysfunction, and an increased pulmonary arterial pressure of 47 mm Hg. The size of the left ventricular chamber was normal, and there was no abnormality in the left ventricular systolic activity, with a left ventricular ejection fraction (LVEF) of 57% (Fig. 3).

The proband underwent cardiac magnetic resonance imaging (MRI) examination after admission and showed mild left ventricular diastolic dilation and left ventricular end-diastolic thickness of 17 mm. T2-weighted imaging showed a non-uniform



**Figure 4.** Electrocardiogram, cardiac magnetic resonance imaging, and T2-weighted cardiac imaging of the proband. (a) The electrocardiogram of the proband shows sinus tachycardia, ST-T changes, and a heart rate of 102 beats per min. (b) Cardiac magnetic resonance imaging shows left ventricular wall thickening, with a diastolic wall thickness of 17 mm. T2-weighted enhanced imaging shows heterogeneous enhancement, the left ventricular myocardial signal is not uniform, there is scattered patchy hyperintensity, and there is enhancement of the left ventricular anterior wall and anterior septum, which shows patchy enhancing lesions (arrow). (c) Diffusion-weighted imaging shows left ventricular myocardial hyperintensity and a lack of uniform distribution. The anterior wall, inferior wall, and lateral wall of the left ventricle and the interventricular septum are seen to be involved (arrow)

left ventricular myocardial signal, with T2-weighted scattered patchy hyperintensity. Diffusion-weighted imaging showed a slight increase in intensity of the signal in the left ventricular myocardium. However, the imaging signal distribution was uneven, located in the anterior wall, inferior wall, lateral wall, and interventricular septum. Cardiac MRI findings were consistent with cardiomyopathy (Fig. 4).

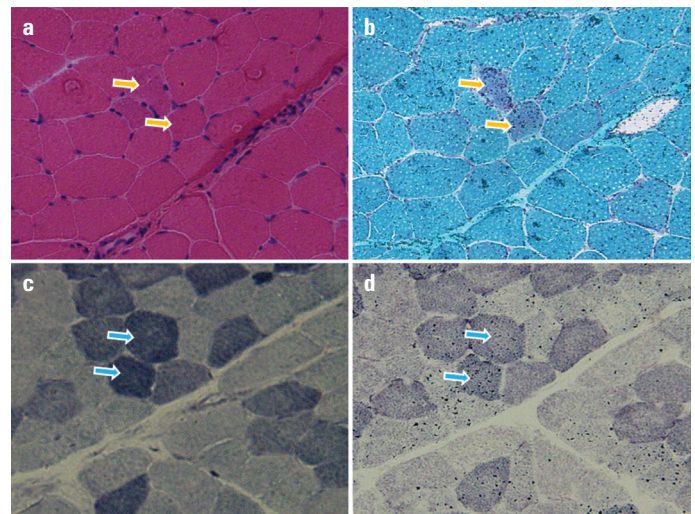


**Figure 5.** The <sup>99m</sup>Tc-macro aggregated albumin perfusion single-photon emission computed tomography images and lung function studies in the proband. (a, b) The images show that both lungs show no abnormalities, and that the radioactive signal is weak with no defective areas. The <sup>99m</sup>Tc-macro aggregated albumin perfusion single-photon emission computed tomography images show clear development of both lungs, with normal ventilation-perfusion matching. (c) The lung function studies show normal to moderate decrease in lung capacity, with a normal residual volume. FEV<sub>1</sub> is normal, with moderate restrictive ventilatory function. FEV<sub>1</sub>, forced expiratory volume in the first second

To exclude respiratory dysfunction and to determine the cause of pulmonary hypertension, pulmonary ventilation function was examined. The results showed normal pulmonary ventilation function. Respiratory pulmonary ventilation/perfusion nuclear imaging showed no signs of pulmonary embolism. Findings of the pulmonary ventilation/perfusion nuclear imaging were consistent with pulmonary hypertension. Examination of the respiratory system showed that the patient had normal respiratory function, indicating that primary pulmonary disease was not the cause of pulmonary hypertension (Fig. 5).

### The proband's sister

The proband's 23-year-old sister had poor motor coordination since childhood, was prone to falls, walked slowly, with a left spinal deformity, and had undergone corrective surgery at 12 years. She had a previous emergency hospital admission for sudden loss of consciousness, difficulty in breathing, and limb weakness. On examination, the patient had no spontaneous breathing, and her ECG showed supraventricular tachycardia. The patient required an endotracheal tube and gradually woke up after ventilation-assisted breathing. Clinical findings included CRP 9.32 mg/mL, troponin 0.04 ng/ml, myoglobin 162.8 ng/mL, LDH 2138 U/L, BNP 973 pg/mL, CO<sub>2</sub> partial pressure 74.3 mm Hg, and O<sub>2</sub> partial pressure 187.0 mm Hg. Echocardiography showed mild mitral regurgitation, but the size of the left ventricular chamber was normal, and there was no abnormality in the left ventricular systolic activity, and the LVEF was 62%. The mother and uncles



**Figure 6.** Photomicrographs of the skeletal muscle biopsy histology. (a) H&E stained skeletal muscle tissue sections (magnification ×200) show that the size of muscle cells is varied, but their shape is regular. Many atrophic skeletal muscle fibers are present, but there are no necrosis or regeneration of muscle cells and no hyperplasia of the muscle fibers. (b) Skeletal muscle biopsy stained with MGT shows “ragged red fibers” (arrow). (c) Skeletal muscle biopsy stained with SDH. (d) Skeletal muscle biopsy stained with NADH-tetrazolium reductase shows a few “ragged blue fibers” (arrow).

H&E - hematoxylin and eosin; MGT - modified Gomori trichrome; SDH - succinate dehydrogenase; NADH - nicotinamide adenine dinucleotide hydrate

of the proband had very poor exercise tolerance and could not engage in heavy physical activity, but their ECG and echocardiography studies were normal.

We detected the causative *MT-ND5* gene mutation of the family with the mutation site c.1315A>G (p.Thr439Ala). Sanger verification showed that both the proband and her maternal members carried the mutation site, which was further confirmed by the clinical diagnosis of mitochondrial cardiomyopathy in this family (Fig. 1b, 1c).

Urine and blood were analyzed by tandem mass spectrometry (MS/MS), showing that elevated levels of lactic acid, 2-hydroxybutyrate, and pyruvate indicated lactic acid disease; 2-keto-valerate, 2-keto-3-methylvalerate, and glutaric acid increased slightly; and a mildly increased glutaryl-carnitine-to-octanoyl-carnitine ratio was found. The results of the blood acid alpha-glucosidase enzyme activity test were normal. These findings were consistent with respiratory chain metabolic dysfunction. Following treatment with myocardial support to improve circulation, vitamin supplements, and other treatments, the patient's symptoms improved (Table 2).

On biopsy of the skeletal muscle of the left biceps for frozen section and histochemical staining, pathological findings showed variation in the size of the muscle cells, with many atrophic fibers and “ragged red fibers” and a small number of “ragged blue fibers”, which was consistent with the diagnosis of mitochondrial myopathy (Fig. 6). In conclusion, combined clinical and laboratory investigations and genetic studies showed a disturbance of respiratory chain metabolism in the proband and relatives.

**Table 2. Laboratory gas chromatography–mass spectrometry analysis result**

Laboratory test	Value	Reference range	Laboratory test	Value	Reference range
Lactic acid–2	270.01 uM	<4.70	Malate -3	4.54 uM	<0.70
Glycolic acid–2	49.43 uM	<2.20	Adipic acid -2	1.33 uM	<5.00
Oxalic acid–2	6.81 uM	<0	3- hydroxy benzoic acid -2	4.49 uM	<0.90
3-Hydroxypropionic acid–2	10.07 uM	<1.10	2- hydroxy adipate -3	6.18 uM	<2.00
Pyruvate-OX–2	285.94 uM	<24.10	3- hydroxy adipate -3	8.34 uM	<3.60
3-Hydroxybutyric acid–2	138.42 uM	<3.70	hexane dicarboxylic acid-2	5.95 uM	<4.70
3-Hydroxyisobutyric acid–2	76.66 uM	<9.00	4- hydroxy phenyl lactic acid -2	47.63 uM	<7.00
2-Hydroxyisovalerate–2	34.02 uM	<0	4- hydroxy pyruvic acid -OX--2	6.75 uM	<0.90
2-Keto-valerate-OX–2	10.53 uM	<0.10	GAA (Ph3.8)	31.48u mol/L/h	>2.88, <98.02
Propionic acid–2	16.95 uM	<2.90	GAA (Ph7.0)	161.15 u mol/L/h	>26.72, <323.31
Urea–2	20.22 uM	>104.60, <763.00	Ala-1	309.75uM	<300.00
3-Hydroxyvalerate–2	4.5 uM	<0	Asp-1	43.35 uM	<80.00
2-Keto-3-methylvalerate–2	13.56 uM	<0	Phe-1	64.48 uM	<120.00
Phosphate–3	112.43 uM	<43.00	C5-OH-1	0.64 uM	<0.60
2-Ketocaproic acid-OX–2	21.28 uM	<0	C5DC-1	0.21 uM	<0.20
Glycine–1	11.34 uM	<0.10	C6-1	0.13	<0.15
Succinate–2	14.45 uM	<65.80	C6:1-1	0.02	<0.10
Glyceryl–3	27.67 uM	<1.60	C18-OH-1	0.04	<0.03
Uracil–2	4.35 uM	<7.00	C5-OH/C2-1	0.02	>0
Glutaric acid–2	9.81 uM	<4.00	C5DC/C8-1	2.89	<2.50
3-Methylglutaric acid–2	2.03 uM	<0	C14:1/C8:1-1	0.65	<2.00

GAA - acid alpha-glucosidase

## Discussion

Mitochondrial cardiomyopathy has only recently been included in the clinical diagnosis and management guidelines in cardiology, but the association with cardiac hypertrophy has been recognized (8). The diagnosis of heritable mitochondrial cardiomyopathy requires a comprehensive clinical evaluation of the patient and family members, biochemical tests, histopathology, and genetic testing.

As shown in this family study, the proband, who had a lung infection that led to acute mitochondrial energy consumption, developed dyspnea, which became more severe, resulting in heart failure and respiratory failure. However, what was unusual in this case was that active antimicrobial treatment did not alleviate the symptoms of heart failure and pulmonary failure, and the patient's echocardiographic and cardiac MRI findings indicated abnormal cardiac function. Blood and urine analyses by MS/MS supported the diagnosis of a metabolic disorder of the respiratory chain due to an inherited metabolic myopathy. This presumptive diagnosis was supported by histological findings on muscle biopsy, including the finding of "ragged red fibers". Genetic analysis detected the mtDNA mutation and confirmed

the diagnosis of mitochondrial cardiomyopathy and hypertrophy, which were further supported when the patient's condition improved following treatment. The present study has demonstrated the importance of understanding the possibility of underlying mitochondrial cardiomyopathy to improve the early diagnosis and treatment of this rare but important condition.

Gene testing has become one of the most reliable methods used in mitochondrial cardiomyopathy research and diagnosis and is helpful in the investigation of family members for detecting gene carriers and for genetic counseling and prenatal diagnosis. However, most patients with genetic mitochondrial cardiomyopathy do not have a definitive genetic diagnosis on clinical presentation. At present, polymerase chain reaction–restriction fragment length polymorphism is used to detect the causative genes (9). However, this method can only be used for specific mutations and has some limitations for the diagnosis of mitochondrial diseases with different mutations.

Recent studies have confirmed that next-generation sequencing (NGS) could be used to detect mitochondrial gene mutations (10). NGS is found to be more efficient, more sensitive, and more accurate (11, 12). Genome sequencing by exon trapping technology and enriched genomic DNA-encoding sequencing

then continues the analysis using NGS, providing WES information, which greatly improves the diagnosis of genetic disease (13, 14). In the present study, NGS was used to detect a novel mutation of *MT-ND5* (c.1315A>G and p.Thr439Ala) in the genome of the proband, and findings were validated in members of the family.

An increasing number of studies have been published on mitochondrial disease caused by *MT-ND5* gene mutations, and among them, c.G13513A is one of the most common point mutations in this gene (15-17). Patients with *MT-ND5* gene mutations often exhibit mitochondrial encephalomyopathy, lactic acidosis, and MELAS and Leigh syndrome (LS) overlap, whereas cardiovascular and respiratory involvement is rare. Mitochondrial disease caused by mutations in the *ND5*-related genes has been reported previously, with the central nervous system and skeletal muscle involvement as the main clinical manifestations (18-21). In the present study, the mitochondrial cardiomyopathy in this family was caused by a novel mutation in the *ND5* gene. The proband and his sister had adult-onset symptoms, with cardiovascular and respiratory involvement at the initial presentation. Heart failure and respiratory failure are usually the main symptoms, with no neurological impairment, which is rare in previously reported mitochondrial diseases.

To our knowledge, this mutation has not been previously reported. The mechanism of the pathogenesis of the *MT-ND5* c.1315A>G mutation remains to be studied. It is possible that this mutation prevents NADH oxidation and mitochondrial proton electrochemical gradient change and increases the formation of oxygen free radicals, resulting in respiratory chain complex I dysfunction and affecting energy metabolism. Increased oxygen free radicals oxidize cell membranes, lipids, proteins, and DNA, and the respiratory chain complex I is also a target of oxygen free radical destruction, thus forming a vicious cycle, eventually leading to cell necrosis. It is possible to hypothesize that the clinical finding reported in the proband with abnormal breathing was mainly caused by an energy metabolism disorder of the respiratory muscles, with abnormalities of the heart due to genetic mitochondrial cardiomyopathy.

### Study limitations

The pathogenic mechanism of this mutation is yet to be further verified on animal models. The patient's complex clinical manifestations are subject to further observation and follow-up to determine the possible interrelationships between them.

### Conclusion

A novel mutation of *MT-ND5* of c.1315A>G was detected in a mitochondrial myopathy family by WES. It provided a basis for the genetic diagnosis of the disease and contributed to understand the disease comprehensively.

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