Barth Syndrome: Different Approaches to Diagnosis

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The diagnosis of Barth syndrome is challenging owing to the wide phenotypic spectrum with allelic heterogeneity. Here we report 3 cases of Barth syndrome with phenotypic and allelic heterogeneity that were diagnosed by different approaches, including whole exome sequencing and final confirmation by reverse-transcription polymease chain reaction. *(J Pediatr 2017;■■:■■-■■).*

Barth syndrome is an X-linked mitochondrial disease characterized by dilated cardiomyopathy or isolated left ventricular noncompaction, cyclic neutropenia, mitochondrial myopathy, short statue, hypocholesterolemia, cognitive dysfunction, and 3-methylglutaconic aciduria.[1,2] However, clinical manifestations of Barth syndrome differ greatly among patients, including variation in cardiac phenotypes, such as dilated cardiomyopathy, isolated left ventricular noncompaction, hypertrophic cardiomyopathy, or endocardial fibroelastosis.[3-5] Thus, Barth syndrome may be underrecognized owing to phenotypic heterogeneity.[6,7] Early diagnosis and treatment improve the prognosis of patients with Barth syndrome; therefore, accurate and prompt diagnosis is necessary.

In addition to phenotypic heterogeneity, allelic heterogeneity is also present in patients with Barth syndrome. Previous studies have revealed various mutations in these patients, including frameshift, nonsense, missense, and splice-site mutations.[8,9] Although direct screening of the *TAZ* gene on the X chromosome is commonly used for patients with clinically suspected Barth syndrome, this fails to identify causative mutations located outside of *TAZ* exonic regions. Therefore, further genetic analysis, such as whole exome sequencing, which detects splicing variants, is required in cases with a negative direct *TAZ* gene screening result but clinical suspicion of Barth syndrome.

Here we report 3 patients with Barth syndrome with phenotypic and allelic heterogeneity, who were diagnosed by different approaches. Clinical, genetic, and biochemical findings for these 3 patients are summarized in the **Table**.

Patient 1

This male patient was born weighing 2.4 kg at 38 weeks gestation. Although the patient showed normal development at 1 month, he presented insufficient weight increase and hypotonia at 4 months. Three weeks later he was referred to a local doctor due to tachypnea, where an enlarged left ventricle with reduced left ventricular ejection fraction (**Figure 1**, A) and hepatomegaly were detected. During transport to Akita University for further investigation, the patient experienced sudden cardiopulmonary arrest; despite resuscitation, he did not recover. Autopsy revealed left ventricular dilatation and endocardial fibroelastosis with thickening of the endocardium (**Figure 1**, B), marked fatty liver, severe congestion of the lung, and myocardium with vacuolar degeneration on light microscopy (**Figure 1**, C). His elder brother was diagnosed with chromosome 10p deletion syndrome, but there was no other relelvant family history.

To investigate the cause of this sudden cardiopulmonary arrest, a screen for mitochondrial disease was performed; enzyme assays of electron transport chain (ETC) activity were performed as described previously[10] using liver and heart tissue. The ETC activity assay revealed decreased complex I activity in both liver and heart tissue.

To genetically screen for mitochondrial disease, whole exome sequencing was performed with the Agilent Sureselect XT All Exon V5 Kit (Agilent Technologies, Santa Clara, California) on an Illumina HiSeq 2500 paired-end reads system (Illumina, San Diego, California) using DNA extracted from liver tissue. The detailed sequencing protocol, including variant calling, has

CL	Cardiolipin
ETC	Electron transport chain
MLCL	Monolysocardiolipin
RT-PCR	Reverse transcription polymerase chain reaction

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Table. Clinical, genetic, and biochemical findings in 3 patients with Barth syndrome

Clinical/genetic/biochemical features	Patient		
	1 (Pt081)	2 (Pt1333)	3 (Pt1401)
Sex	Male	Male	Male
Age of presentation	4 m.o	39 wk gestation	8 m.o
Cardiac phenotype	Dilated form of endocardial fibroelastosis	Diffuse left ventricular hypertrabeculation	Ventricular fibrillation
TAZ mutation	NM_000116:exon8:c.646G>A:p.G216R	NM_000116:c.109 + 6T>G	NM_000116:exon4:c.367C>T:p.R123X
Family history	—	Maternal uncle: endocardial fibroelastosis (died before 1 y.o)	3 mother's cousins: cardiomyopathy (died before 1 y.o)
MLCL/CL ratio (reference range 0-0.3)	Not done	2.6	20
ETC activity (expressed as percentage of citrate synthase activity, organ)	CI (0.6%, heart) CI (2.4%, liver)	Not done	CI, CII, CIV (6.9, 13.8, 17.5%, heart) CI, CIV (0, 7.4%, muscle)

been described previously.[11] Exome sequencing identified a known pathogenic mutation (NM_000116:exon8:c.646G>A:p.G216R).[12,13] The same mutation was identified in the mother's blood. Analysis of urine organic acid and of the monolysocardiolipin/cardiolipin (MLCL/CL) ratio were not performed due to unavailability of samples.

Patient 2

This male patient was born at 39 weeks gestation by emergency cesarean delivery at Toyama University Hospital (Apgar score of 5/7) because of decreased variability on the nonstress test, left and right heart failure, and functional pulmonary atresia by echocardiography. Echocardiography during the fetal period showed diffuse left ventricle hypertrabeculation (**Figure 1**, D). Severe right ventricle failure was present at birth (**Figure 1**, D), which was improved by continuous administration of prostaglandin E1. However, cardiac shock from left-sided heart failure occurred on day 4 after birth. With cardiopulmonary support, his left ventricular systolic function gradually improved, and at 3 months the heart failure was well controlled by oral medications. At age 19 months, the patient exhibited normal growth and mildly decreased intellectual ability. His echocardiogram showed diffuse left ventricular hypertrabeculation and decreased ejection fraction.

The patient's family history revealed that a maternal uncle had died during infancy due to endocardial fibroelastosis. Laboratory tests revealed an elevated lactic acidosis/pyruvic acid ratio (37.4). Analysis of urine organic acid found no elevation of 3-methylglutaconic acid or 3-methylglutaric acid. Direct sequencing of the *TAZ* gene and other sarcomere protein genes were all negative. Whole exome sequencing was performed using blood DNA to screen for mitochondrial cardiomyopathy as described above, and a novel mutation was identified in an intronic region of *TAZ* (TAZ:NM_000116:c.109 + 6T>G). The same mutation was identified in the mother. Although the

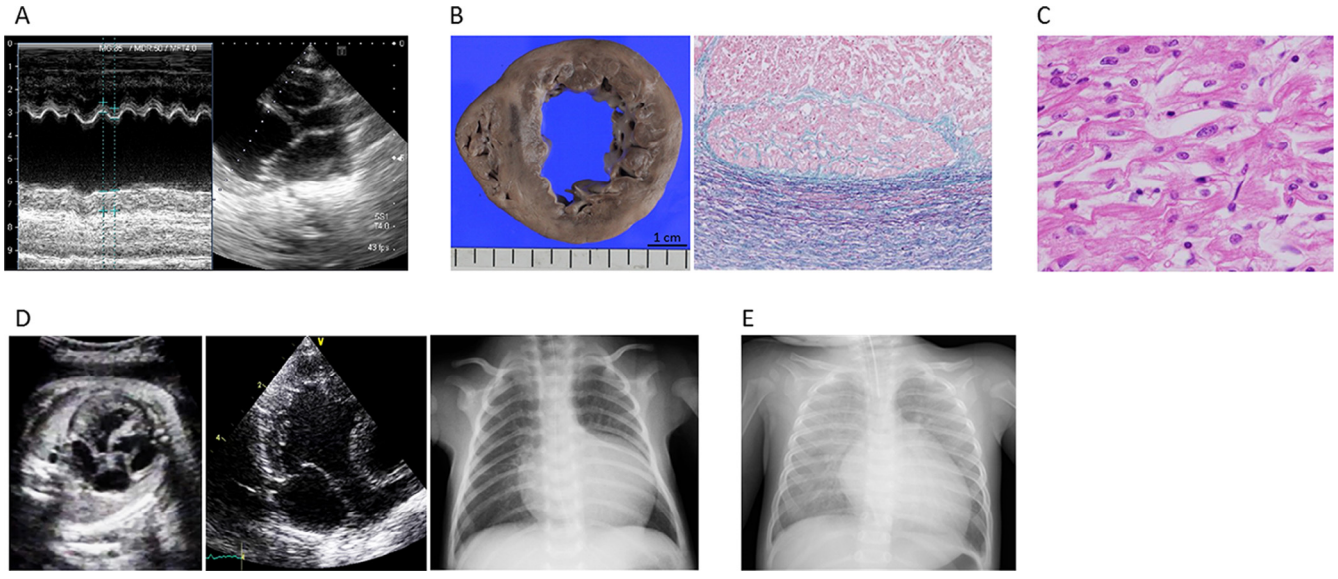

Figure 1. Imaging findings in 3 patients with Barth syndrome. Patient 1: **A,** enlarged left ventricle with reduced left ventricular ejection fraction on echocardiography, **B,** left ventricular dilatation and endocardial fibroelastosis with thickening of the endocardium at autopsy of the heart, and **C,** myocardium with vacuolar degeneration on light microscopy. **D,** Patient 2: diffuse left ventricle hypertrabeculation on echocardiography in the fetal period and at birth, and enlarged heart on chest X-ray at birth. **E,** Patient 3: enlarged heart on chest X-ray on arrival at the hospital.

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