CASE REPORT

NGS identifies *TAZ* mutation in a family with X-linked dilated cardiomyopathyElim Man,¹ Katherine A Lafferty,² Birgit H Funke,² Kin-Shing Lun,¹ Shu-Yan Chan,¹ Adolphus Kai-Tung Chau,¹ Brian Hon-Yin Chung¹¹Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong²Laboratory for Molecular Medicine, Partners HealthCare Center for Personalized Genetic Medicine, Cambridge, Massachusetts, USA

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SUMMARY

We reported a family with two male siblings affected with infantile dilated cardiomyopathy (DCM). Extensive evaluation failed to identify the underlying cause for the DCM. Next generation sequencing (NGS) with targeted enrichment identified a hemizygous variant c.718G>C (p.Gly240Arg) in the *TAZ* gene. This variant has been reported in three other families with X linked infantile DCM and is therefore likely pathogenic. NGS allows efficient screening of a large number of uncommon genes in complex disorders like DCM, in which there is substantial genetic and phenotypic heterogeneity. The identification of *TAZ* mutation has major impact on their medical care as the surveillance needs to be expanded to cover for the Barth syndrome, a severe metabolic phenotype also caused by *TAZ* mutation, in addition to DCM.

BACKGROUND

Familial dilated cardiomyopathy (DCM) is defined as DCM of unknown cause in two or more closely related family members.¹ DCM is a genetically heterogeneous group of heart disorder of largely unknown aetiology. At least 40 different genes were implicated in the pathogenesis of this disorder.² Comprehensive and accurate genetic testing of all causative genes inevitably involves enormous amount of DNA sequencing and the conventional Sanger sequencing approach is both time- and cost-consuming. With the employment of next-generation sequencing (NGS) technology in clinical practice, specific genetic diagnosis can be made in a highly efficient manner.³ In this report, we report two Chinese male siblings diagnosed with DCM in their childhood and were found to have a pathogenic *TAZ* gene mutation using the NGS technology.

CASE PRESENTATION

YHL (*Patient III4*, figure 1) was born at full term by normal vaginal delivery in China. His antenatal course was uneventful. He presented with perioral cyanosis on the second day of life. His first chest radiography showed cardiomegaly. Echocardiogram showed left ventricular hypertrophy and dilatation (figure 2A). He was suspected to have myocarditis due to intrauterine infection. However, microbiological studies were all unrevealing. He was started on digoxin and captopril. Serial yearly echocardiogram showed progressive dilatation of the left heart and decline of cardiac function. His general condition and growth velocity remained normal. Cardiac

catheterisation later confirmed the diagnosis of DCM and poor left ventricular contractions. The histology of the endomyocardial biopsy, however, did not reveal any obvious aetiology. Multigated acquisition showed decreased left ejection fraction and mild global hypokinesia (figure 2B). His clinical condition remained stable while he was on the antiheart failure medications.

YML (*patient III5*, figure 1), his younger brother, was born 7 years later. Fetal ultrasound performed during the antenatal period at 16 weeks and 35 weeks of gestation were unremarkable. YML was born at full term by an elective lower caesarean section for breech presentation. His perinatal course was uneventful. He thrived well with normal development appropriate for his age. He remained asymptomatic until he was incidentally found to have cardiomegaly during hospitalisation for viral bronchiolitis at 5 months of age. He was not dysmorphic and there was no evidence of muscle weakness. Electrocardiogram showed sinus tachycardia with no abnormal ST segment changes. Creatine kinase (CK) and troponin T level were normal. Echocardiogram showed dilated left heart chambers and impaired ventricular contractility. A small rim of pericardial effusion was also detected. He was managed as acute myocarditis in the intensive care unit. He required assisted mechanical ventilation and high inotropic support. Intravenous immunoglobulin infusion (2 mg/kg/dose) was empirically given once. His clinical condition and cardiac function gradually improved by the end of first week after admission. He was able to wean-off inotropic support and extubated by the third week of admission. He was finally discharged from the hospital after 3 weeks of treatment. The diagnosis was acute decompensated heart failure of DCM as there was no significant elevated troponin level. His clinical condition remained stable during subsequent follow-up while on digoxin, captopril

Figure 1 Pedigree of the indexed family.

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Figure 2 (A) YHL's chest x-ray and echocardiogram showing severe cardiomegaly. (B) Multigated acquisition scan showed decreased left ejection fraction of 40% with mild global hypokinesia.

and carvedilol. His serial echocardiogram also showed improvement in left ventricular contractions.

There was otherwise no family history of congenital heart disease or premature deaths and his parents were both healthy. However, further enquiry revealed that their first son (III3, figure 1), in fact, died at the age of 8 months in Mainland China within 4 days of hospitalisation during an episode of suspected pneumonia. Echocardiograms of the parents were normal with no evidence of DCM and CK level was checked to be normal in the mother. As the two brothers were likely affected by the same condition, an X linked mode of inheritance was considered but other modes of inheritance could not be ruled out. Both YHL and YML did not suffer from any recurrent infections or neutropaenia. Metabolic workup, including urine for 3-methylglutaconic acid, was also unrevealing. Array comparative genomic hybridisation by NimbleGen CGX-12 in YML was normal.

INVESTIGATIONS

Oligonucleotide-based target capture (Sureselect, Agilent) followed by NGS (Illumina HiSeq2000) on the patient's DNA samples was used to capture variants of 46 genes implicated in the causation of cardiomyopathy (Partners Healthcare Center for Personalized Genetic Medicine). Clinically significant or novel variants are confirmed by independent Sanger sequencing. Variants classified as likely benign or benign are not confirmed. Variant calls are generated using the Burrows-Wheeler Aligner followed by Genome Analysis ToolKit analysis. A hemizygous variant c.718G>C (p.Gly240Arg) in exon 10 of the *TAZ* gene in YML was identified and confirmed by Sanger sequencing. This variant was also detected in YHL and their mother (figure 3).

Figure 3 Gly240Arg variant on the *TAZ* gene in our proband YML, his elder brother YHL and his mother identified by (A) next generation sequencing, (B) Sanger sequencing.

DISCUSSION

Familial DCM is a phenotypically and genetically heterogeneous group of cardiac disorder. Unlike other types of cardiomyopathies, the pathogenesis of DCM involves more diverse pathways involving genes encoding for various cellular components (eg, sarcomere, Z-disk, nuclear lamina proteins, intermediate filaments and dystrophin-associated glycoprotein complex, etc.) and more than 40 genes have been implicated (appendix).²⁻⁴ Each of these genes provides a very modest contribution to the overall incidence and many involve rare and private mutations in isolated families. The conventional mutation pick-up rates for DCM are no more than 30% overall in adult studies.^{5,6} Due to such low sensitivity, genetic testing of DCM is often largely hindered by the timely and financially demanding efforts required in screening all known disease genes.⁷ Many a time, only limited genes could be tested using the conventional sequencing approach.

With increasing employment of NGS in clinical practice, specific genetic diagnosis can be made in a more comprehensive and affordable way. This technology allows simultaneous interrogation of millions of DNA base pairs in a single run. As a

Table 1 Phenotypes of patients with Gly240Arg variant on the TAZ gene^{8–10}

Family	Patient	Age of onset (months)	Diagnosis	Clinical severity	Age of death (months)	Other features	References
A	1	—	DCM	HF, died	3–4	—	D'Adamo <i>et al</i> ¹⁰
	2	—	DCM	HF, died	3–4	—	
	3	—	DCM	HF, died	3–4	—	
	4	1	DCM	HF	Living	No	
B	1	108	DCM	HF	Living	No	Bisser <i>et al</i> ⁸
	2	60	DCM	HF	Living	No	
C	1	3	HDCM	HF, died	3	No	Chen <i>et al</i> ⁹
	2	4	EFE	HF, died	4	No	
D*	1	At birth	DCM	HF	Living	No	This study
	2	5	DCM	HF	Living	No	

*Index family.

EFE, endocardial fibroelastosis; HDCM, hypertrophic dilated cardiomyopathy.

result, efficient screening of a large number of uncommon genes in complex disorders like DCM, in which there is substantial overlap among phenotypes and some mutations are associated with more than one phenotype is feasible within a reasonable time frame.

In our family, NGS technology with targeted enrichment enhances sequence coverage and screening efficiency of the genes of interest. The coverage of the 46 cardiomyopathy genes is 300–400-fold in our patient in one single run and any base that has less than 20× coverage is considered failed and Sanger-sequenced to fill in the missing data. Again, if a variant is identified it will be confirmed with a second Sanger assay. This approach detects 100% of substitution variants (95% CI=82 to 100) and 95% of small insertions and deletions (95% CI 98.5 to 100) in the coding regions of the genes. However, it does not detect all variants in non-coding regions that could affect gene expression or copy number changes encompassing a large portion of the gene. Not much published data are available yet in the literature regarding the detection rates of DCM in the paediatric population using NGS technology. Nonetheless, preliminary data from the reference laboratory we use suggest an overall detection rate of up to 40% for DCM using this approach (<http://pcpgm.partners.org/lmm/tests/cardiomyopathy>).

TAZ (taffazzin) gene is located at chromosome Xq28 and it is highly expressed in cardiac and skeletal muscles. Variants on the TAZ gene are known to have high genetic heterogeneity and have been reported in families with Barth syndrome, DCM, left ventricular non-compaction and endocardial fibroelastosis (EFE). Barth syndrome, also known as 3-methylglutaconic aciduria type II (MGCA2), is a severe metabolic disease caused by the mutations of the TAZ gene. It is characterised by DCM or EFE presented in infancy, growth retardation, developmental delay, neutropaenia, skeletal myopathy, abnormal cholesterol metabolism, lactic acidosis and abnormal 3-methylglutaconic aciduria.

Gly240Arg variant on the exon 10 of the TAZ gene detected in our patients is likely pathogenic as it has been reported in three other families with X linked infantile DCM (table 1).^{8–10} All reported patients with the Gly240Arg variant (including ours) do not have any clinical features like neutropaenia, increased 3-methylglutaconic aciduria, skeletal myopathy or stunted growth, suggestive of Barth syndrome. Nevertheless, the identification of specific TAZ mutation poses major impact on the medical surveillance of our patients as they need to be monitored for symptoms of Barth syndrome in addition to DCM which may present later in life.

Similar to DCM, many complex and heterogeneous childhood diseases are well known to involve a number of causative

yet uncommon genes in their pathogenesis. In the era of NGS technology, genome sequencing being an efficient and affordable tool for genetic diagnosis of complex childhood diseases is no longer too far from reach. Its value does not only limit to resolving the genetic basis but also shedding light on guiding treatment and predicting disease prognosis in a gene-specific and even variant-specific context in the near future.

Learning points

- Familial dilated cardiomyopathy DCM is a complex and heterogeneous group of cardiac disorders with many uncommon genes implicated in the pathogenesis.
- Next-generation sequencing technology facilitates more efficient and affordable genetic diagnosis of complex childhood diseases like familial DCM.
- Accurate genetic diagnosis of familial DCM is vital for the provision of comprehensive medical care and effective genetic counselling, as exemplified by our family with TAZ mutation.

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Competing interests None.

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