Clinical utility of exome sequencing in infantile heart failure

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Purpose: Pediatric cardiomyopathy is rare, has a broad differential diagnosis, results in high morbidity and mortality, and has suboptimal diagnostic yield using next-generation sequencing panels. Exome sequencing has reported diagnostic yields ranging from 30% to 57% for neonates in intensive care units. We aimed to characterize the clinical utility of exome sequencing in infantile heart failure.

Methods: Infants diagnosed with acute heart failure prior to 1 year old over a period of 34 months at a large tertiary children's hospital were recruited. Demographic and diagnostic information was obtained from medical records. Fifteen eligible patients were enrolled.

Results: Dilated cardiomyopathy was the predominant cardiac diagnosis, seen in 60% of patients. A molecular diagnosis was identified in 66.7% of patients (10/15). Of those diagnoses, 70%

would not have been detected using multigene next-generation sequencing panels focused on cardiomyopathy or arrhythmia disease genes. Genetic testing changed medical decision-making in 53% of all cases and 80% of positive cases, and was especially beneficial when testing was expedited.

Conclusion: Given the broad differential diagnosis and critical status of infants with heart failure, rapid exome sequencing provides timely diagnoses, changes medical management, and should be the first-tier molecular test.

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INTRODUCTION

Pediatric cardiomyopathy is a rare condition of the myocardium occurring in approximately 4.4 in 100,000 infants per year.^{1–3} Dilated cardiomyopathy (DCM) is the most common subtype affecting the pediatric population, followed by hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy, and then a mixed or other cardiomyopathy phenotype.³ In one study, 46% of patients with pediatric DCM progressed to death or transplant within the first 5 years of diagnosis, highlighting the often devastating outcomes of pediatric cardiomyopathy.¹ The etiologies for pediatric patients with cardiomyopathy are varied and include infections, metabolic diseases, syndromic diagnoses, nonsyndromic familial cardiomyopathy, and idiopathic cardiomyopathy.^{4–8}

Yield of genetic testing varies widely based on the phenotype, age of presentation, and type of testing pursued. Many recent studies have demonstrated a diagnostic yield between 33% and 58% of patients who received exome or

genome sequencing for any indication while in a neonatal intensive care unit or while critically ill.^{10–14} Exome and genome sequencing provide rapid results for patients and families and also change medical management in up to half of cases.^{11,13} Next-generation sequencing panels identify a genetic diagnosis in 10–60% of cardiomyopathy cases, depending on the subtype and cohort.⁵ In one cohort of patients with pediatric dilated cardiomyopathy, multigene panel testing yielded a diagnosis in 54% of patients whereas exome sequencing identified a genetic diagnosis in 87% (13/15) of probands.⁹ Herein, we report the experience of a large tertiary children's hospital and cardiac transplant center with exome sequencing in neonates and infants with acute presentations of heart failure.

MATERIALS AND METHODS

Eighteen patients presented to our institution with heart failure within the first year of life and were evaluated by

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metabolic and genetics physicians as part of their clinical care between June 2016 and May 2019. Participants were consented into one of two institutional review board (IRB)-approved pediatric genomic sequencing studies at CHOP (IRB #16-013231 and IRB #18-014911) for this prospective observational study. These protocols include permission for publication of de-identified patient data. All appropriate consent and protocol forms have been archived. Three patients (two with DCM, one with HCM) were not enrolled due to parental decision to not participate in this study. Exclusion criteria included anatomic congenital heart disease sufficient to cause the degree of heart failure and age at presentation of greater than 12 months.

Clinical genetic testing recommendations were made by the clinical team per standard of care, and included either standard or rapid exome sequencing with or without concurrent mitochondrial sequencing. Standard exome sequencing was performed by GeneDx (Gaithersburg, MD) or the Children's Hospital of Philadelphia's Division of Genomic Diagnostics (Philadelphia, PA). All mitochondrial sequencing and rapid exome sequencing were performed by GeneDx. XomeDxXpress was utilized for its rapid turnaround time of verbal result in 7 days and written report in approximately 2 weeks in nine cases. All testing was trio based, including both parents, with the exception of patient 12 whose father was unavailable for testing. Pertinent demographic, diagnostic, and outcomes data were abstracted from their medical records.

Genetic testing was considered diagnostic when a likely pathogenic or pathogenic variant(s) were identified in gene(s) associated with the patient's clinical features. In two cases, variants of uncertain significant (VUS) were included in the diagnostic category, as the patients had extracardiac features strongly consistent with these syndromic diagnoses (Table 1). One patient had a homozygous VUS in *PPCS* and had other features of *PPCS* deficiency including profound hypotonia and elevated creatine kinase levels. Another patient, who already carried a clinical diagnosis of Noonan syndrome, had one VUS in *LZTR1*, a Noonan syndrome gene.

Change in management for probands was defined as initiating targeted treatment based on genetic testing, consultation of other pertinent subspecialties, or redirection of care. Change in management for family members was defined as identifying genetic predisposition to cardiomyopathy in family members without a known history of cardiomyopathy, change in recurrence risk counseling, or clearance of family members from cardiac surveillance.

RESULTS

Fifteen patients with infantile heart failure who had exome sequencing recommended as part of their diagnostic evaluation were enrolled (Table 1). Of those, 60% of patients presented with dilated cardiomyopathy (Fig. 1a). The median age of diagnosis of cardiomyopathy was 10 days (range 0 days to 8 months). Seven patients (46.7%) were diagnosed prenatally or within the first 48 hours of life (Fig. 1b). Patient

11 was the only with a first- or second-degree relative with cardiomyopathy prior to the patient's presentation. Rapid exome sequencing was sent for nine patients (60%), with a median written report turnaround time of 10 days (range 9–14). Standard exome sequencing in the remaining six patients had a median turnaround time of 42 days (range 29–71). Nine of 15 (60%) had mitochondrial DNA sequencing at the time of exome sequencing, all of which was normal. At the time of publication, 9/15 (60%) patients were alive. All six deceased patients died as a result of redirection of care, either due to clinical status or genetic diagnosis. Two patients underwent successful cardiac transplantation.

Exome sequencing was diagnostic in 10/15 (67.7%) patients (Table 1; Supplementary Table 1). Seventy percent of these diagnoses would have been missed using standard cardiomyopathy panels at the majority of commercial laboratories investigated. All six patients with extracardiac manifestations prior to testing received diagnoses via exome sequencing. Four of nine without extracardiac manifestations received diagnoses. Four metabolic or mitochondrial cardiomyopathies were identified: *NDUF11*-related disorder (OMIM 300403), *ACAD9* deficiency (OMIM 611103) *PPCS*-related cardiomyopathy (OMIM 609853), and mitochondrial trifunctional protein deficiency (OMIM 609015). Four syndromic diagnoses were identified: Noonan syndrome (OMIM 600574), Alström syndrome (OMIM 203800), X-linked agammaglobulinemia (OMIM 300705), and *NAA15*-related neurodevelopmental disorder (OMIM 608000). Only two of ten diagnoses were in primarily cardiac related genes: *MYH7* (OMIM 613426) and *FLNC* (OMIM 102565).

Exome sequencing results changed the patient's medical management in eight cases (53.3%) and changed familial management in two-thirds of cases. Three families chose to reorient care goals away from aggressive life support, such as extracorporeal membrane oxygenation, based on the genetic testing results and prognosis. In the case of *ACAD9* deficiency, riboflavin supplementation was initiated in conjunction with other cardiac medications with subsequent resolution of his severe ventricular dysfunction and dilation, and improvement in left ventricular hypertrophy. For syndromic diagnoses, multiple subspecialist evaluations were initiated.

DISCUSSION

Exome sequencing in infantile heart failure has a high diagnostic yield (67%) in our cohort and impacted medical management in all diagnostic cases. Genes for 70% of diagnoses were not on cardiomyopathy panels and would have been missed without the use of exome sequencing. Extracardiac manifestations may have been challenging to identify in critically ill neonates for many of the syndromic diagnoses identified in our cohort. As such, exome sequencing was critical for making a diagnosis and ensuring proper care, regardless of time to result, though additionally so for rapid testing. For example, patient 9 was a healthy infant girl who presented with acute decompensated heart failure at 2 months

Table 1 Exome sequencing results in infants with acute heart failure

Patient	Age at diagnosis (days)	Cardiac diagnosis	Extracardiac features	Exome type	Gene	Variant	Classification	Diagnosis
Patient 1	2	DCM	Microcephaly, hypoglycemia	Rapid trio	NDUFB11	c.262 C>T, p.R88X	Pathogenic	NDUFB1-related disorder
Patient 2	212	HCM	vPS, DD, short stature	Standard trio	LZTR1	c.1165C>T; p.L389F	VUS	Noonan syndrome
Patient 3	0	HLHS + LVNC		Rapid trio	MYH7	c.1275_1277delGGCinsAGA; p.A426E	Likely pathogenic	MYH7-related cardiomyopathy
Patient 4	243	HCM	Lactic acidosis, FTT, DD	Standard trio	ACAD9	c.1240C>T; p.Arg414Cys	Likely pathogenic	ACAD9 deficiency
Patient 5	0	HLHS + LVNC		Rapid trio	PRKAG2	c.698C>G; p.A233G	VUS	None
Patient 6	35	DCM		Rapid trio + mito	n/a	n/a	n/a	None
Patient 7	0	DCM		Rapid trio + mito	LAMA4	c.503G>A; p.R168K	VUS	None
Patient 8	10	DCM		Rapid trio	FLNC	c.4021C>T; p.R1341X	Likely pathogenic	FLNC-related cardiomyopathy
Patient 9	30	DCM	After diagnosis: early stage retinal dystrophy	Rapid trio + mito	NFU1 TDGF1	c.452delT, p.L151RfsX22 c.275G>A, p.G92E	VUS	
Patient 10	76	Myocarditis		Rapid trio + mito	ALMS1	c.928C>T, p.Q310X c.3016_3017delCA, p.H1006X	Pathogenic	Alström syndrome
Patient 11	0	DCM		Rapid trio + mito	BTX	c.1178-1G>A; IVS13-1G>A	Pathogenic	X-linked agammaglobulinemia
Patient 12	0	DCM		Rapid trio + mito	HCM4	c.2330T>G; p.Leu777Arg	VUS	None
Patient 13	64	DCM	Respiratory insufficiency, hypotonia, elevated CK	Standard trio + mito	TNNI3K	c.1437delA; p.G480DfsX7	VUS	None
Patient 14	79	HCM	Low set ears, overfolded helices, eczema, developmental delay	Standard trio + mito	PPCS	c.317G>C; p.Arg106Pro	VUS	PPCS-related disorder
Patient 15	1	DCM	Elevated long-chain species on acylcarnitine profile, lactic acidosis, elevated CK	Rapid trio + mito	LMNA	c.1685T>A; p.Leu562His	VUS	NAA15-related disorder
					NAA15	c.1009_1012delGAAA; p.Glu337fs	Pathogenic	
					HADHB	c.1228_1240del; p.G410WfsX41	Likely pathogenic	Trifunctional protein deficiency
						c.181C>T; p.R61C	Likely pathogenic	

CK creatine kinase, DCM dilated cardiomyopathy, DD developmental delay, FTT failure to thrive, HCM hypertrophic cardiomyopathy, HLHS hypoplastic left heart syndrome, LVNC left ventricular noncompaction, vPS valvular pulmonic stenosis, VUS variant of uncertain significance.

Fig. 1 Patient characteristics. (a) Cardiac phenotype and (b) age at diagnosis for 15 infants presenting with heart failure. *DCM* dilated cardiomyopathy, *HCM* hypertrophic cardiomyopathy, *HLHS* hypoplastic left heart syndrome, *LVNC* left ventricular noncompaction.

of age and was identified to have Alström syndrome via rapid exome sequencing. Not only were results returned in a timely manner allowing the intensive care team to provide prognostication information for the family, but also other subspecialists were involved for the syndromic nature of this disease. The child has since been noted to have cone-rod dystrophy, photophobia, nystagmus, and elevated triglycerides. These manifestations of the disease were not identified until targeted subspecialist screening was initiated due to the molecular diagnosis. Additionally, her mother was able to have targeted genetic testing via chorionic villus sampling for her ongoing subsequent pregnancy, which confirmed the fetus was unaffected.

The diagnoses identified in this cohort demonstrate the wide clinical spectrum of diagnoses that can present with infantile heart failure, notably metabolic and syndromic diagnoses in the majority of patients with diagnostic exome sequencing. This highlights the importance of genetics and metabolism specialists in the diagnostic work-up for patients with infantile heart failure. While this study is limited by a relatively small cohort size at one medical center, infantile heart failure due to cardiomyopathy is a rare disease and diagnostic yields were consistent with other larger studies of

exome sequencing in neonatal or pediatric intensive care units.^{10–14} Further studies including cost–benefit analysis and multicenter prospective studies may help refine the diagnostic yield and clinical utility of rapid exome sequencing in this unique population. In conclusion, given the broad differential diagnosis and critical status of infants with heart failure, rapid exome sequencing provides timely diagnoses, changes medical management, and should be the first-tier molecular test for infantile heart failure.

SUPPLEMENTARY INFORMATION

The online version of this article (<https://doi.org/10.1038/s41436-019-0654-3>) contains supplementary material, which is available to authorized users.

DISCLOSURE

J.J., D.C. and S.Y. are employees of GeneDx, Inc. The other authors declare no conflicts of interest.

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