The Impact of Rapid Exome Sequencing on Medical Management of Critically Ill Children

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Objectives To evaluate the clinical usefulness of rapid exome sequencing (rES) in critically ill children with likely genetic disease using a standardized process at a single institution. To provide evidence that rES with should become standard of care for this patient population.

Study design We implemented a process to provide clinical-grade rES to eligible children at a single institution. Eligibility included (a) recommendation of rES by a consulting geneticist, (b) monogenic disorder suspected, (c) rapid diagnosis predicted to affect inpatient management, (d) pretest counseling provided by an appropriate provider, and (e) unanimous approval by a committee of 4 geneticists. Trio exome sequencing was sent to a reference laboratory that provided verbal report within 7-10 days. Clinical outcomes related to rES were prospectively collected. Input from geneticists, genetic counselors, pathologists, neonatologists, and critical care pediatricians was collected to identify changes in management related to rES.

Results There were 54 patients who were eligible for rES over a 34-month study period. Of these patients, 46 underwent rES, 24 of whom (52%) had at least 1 change in management related to rES. In 20 patients (43%), a molecular diagnosis was achieved, demonstrating that nondiagnostic exomes could change medical management in some cases. Overall, 84% of patients were under 1 month old at rES request and the mean turnaround time was 9 days.

Conclusions rES testing has a significant impact on the management of critically ill children with suspected monogenic disease and should be considered standard of care for tertiary institutions who can provide coordinated genetics expertise. *(J Pediatr 2020;226:202-12)*.

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Exome sequencing is the simultaneous sequencing of all approximately 20 000 genes in the human genome and is increasingly a first-line diagnostic test for children with multiple congenital anomalies, complex neurodevelopmental phenotypes, and other likely monogenic disorders.[1,2] Numerous studies have demonstrated that exome sequencing provides a definitive molecular diagnosis in 30%-50% of children with these phenotypes.[3-6] However, exome sequencing is not yet in broad use in pediatric and neonatal intensive care units (IUCs), despite the fact that these patients are enriched for genetic disease.[4-15] Barriers to the widespread adoption of exome sequencing in the ICU setting include the impression that the turnaround time is too long to be useful in the critical care setting; complex test logistics, which requires pretest genetic

CMA	Chromosomal microarray
CNV	Copy number variant
ECMO	Extracorporeal membrane oxygenation
ICU	Intensive care unit
rES	Rapid exome sequencing
RIGhT	Rapid Inpatient Genomic Testing
SNV	Single nucleotide variant
VUS	Variant of uncertain significance

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counseling and may require obtaining samples from both parents; high test costs with poor reimbursement; and just emerging data on how exome sequencing impacts clinical management of these children.[16-20]

We describe our experience developing the Rapid Inpatient Genomic Testing (RIGhT) study, a clinical program for rapid exome sequencing (rES) within neonatal, pediatric, and cardiac ICUs at a single institution. This program was developed as a part of routine clinical care and was not subsidized by research or other funds. Rather than focusing on the diagnostic yield of rES, we evaluated how the results were used by ICU physicians to change medical and surgical management of these critically ill children.

Methods

This study was performed at Seattle Children's Hospital. This hospital has a 32-bed neonatal ICU that sees approximately 500 patients per year, a 38-bed pediatric ICU that sees approximately 2000 admissions per year, and a 20-bed cardiac ICU that sees approximately 600 admissions per year. This study was approved by the Seattle Children's Hospital Institutional Review Board (Activity ID: CR00003151, Institutional Review Board ID: STUDY00000553).

Study Design and Participants

The RIGhT study began in October 2016. Data are reported through July 2019. For a patient to be enrolled in the study, the intensivist first consulted medical or biochemical genetics. All geneticists and intensivists were made aware of the study in October 2016. Recommendations for rES ultimately were made by the consulting geneticist, although the intensive care team and consulting geneticist engaged in collaborative decision making. Initial inclusion criteria were (1) consultation with a geneticist, (2) suspected monogenic disorder, (3) likelihood of rapid diagnosis altering management, (4) age less than 6 months and critically ill in the ICU, (5) availability of both biological parents for trio sequencing, and (6) previous negative chromosomal microarray (CMA). The send out laboratory (GeneDx) performing the sequencing was only able to offer rapid testing for trio sets. If 1 or both parents were unavailable, testing with an approximately 4-week turnaround time was made available outside of this study. In January 2017, the inclusion criteria were broadened to include children of all ages in an ICU and the prerequisite for CMA was eliminated. The revisions were made based on observations that requiring CMA delayed diagnosis in some patients, the reference laboratory performing exome sequencing could detect CNVs of at least 3 exons, and monogenic disease presents in children older than 6 months.[21]

RIGhT Committee Review Process

Referring board-certified geneticists identified the indication for testing, generated a phenotype-driven list of candidate genes, suspected mode of inheritance, and proposed changes in clinical management based on results. Referral information was captured on a standardized 2-page form (**Appendix**; available at www.jpeds.com). The major test indication as well as other phenotypic features were recorded using human phenotype ontology terms. A laboratory genetic counselor provided an initial review of whether the cases met the inclusion criteria. If the case was appropriate, it was then reviewed by a committee of board-certified geneticists with a variety of expertise, including dysmorphology, epilepsy, biochemical genetics, vascular and lymphatic disorders, and mosaicism. The committee provided review within 1 business day and unanimous approval was required to proceed.

Genetic Counseling and Informed Consent

Two clinical genetic counselors were appointed to provide in-person pretest counseling and all families provided informed consent for clinical exome sequencing. All patients and families were given the option of receiving secondary findings. A clinical administrator coordinated registration of both parents and sample collection logistics.

Sample Collection and Sequencing

Peripheral blood samples from the patient and both biological parents were collected and shipped to the laboratory. In cases in which mitochondrial DNA sequencing was required and the patient had undergone a recent red blood cell transfusion, a buccal swab was collected. The laboratory also received a pedigree and the consultant geneticist's notes.

Trio exome sequencing was performed by a send out laboratory (GeneDX) that provides a verbal preliminary result within 10 calendar days of receipt of samples. Sequencing was done using the Agilent Clinical Research Exome kit (Agilent, Santa Clara, California). Targeted regions were sequenced simultaneously on an Illumina HiSeq (Illumina, San Diego, California) with 100-bp paired end reads. The bidirectional sequence was assembled and aligned to human reference genome build GRCh37/UCSC hg19 and analyzed for sequence variants using a custom developed analysis tool (Xome Analyzer; GeneDx, Gaithersburg, Maryland).[22]

Return of Results and Multidisciplinary Case Review

Preliminary verbal results were returned to the consulting geneticist who was then responsible for informing the critical care team and family. Final results were scanned into the electronic health record and returned by the consulting geneticist and/or genetic counselor. Cases were classified as molecularly diagnosed when pathogenic or likely pathogenic variant(s) were detected in a gene that explained the patient's phenotype.[23] Cases in which a pathogenic or likely pathogenic variant explained part, but not all, of the patient's phenotype were classified as partial diagnoses. Cases were classified as uncertain when there was any variant that was potentially related to patient's phenotype but there was insufficient evidence to be certain. Cases were classified as nondiagnostic when no disease-associated variants were identified.

All cases were reviewed at monthly multidisciplinary team meetings that included geneticists, genetic counselors, pathologists, and neonatologists. Pediatric critical care physicians reviewed cases as needed. Pertinent dates, molecular results, and changes in management were recorded in a database. Any change in medication (initiation or discontinuation), laboratory testing, surgical plan, or imaging plan was categorized as a discrete instance of management change. A single patient could have more than 1 management change related to rES.

All cases were also retrospectively reviewed to confirm previously documented changes in management as well as evaluate for any additional changes in management by a single person. Two neonatologists also independently reviewed each neonatal ICU case. One pediatric intensivist reviewed all pediatric and cardiac ICU cases. Any discrepancies were reviewed by the ICU attending and consulting geneticist of record for the case. Only if both attending physicians agreed that the rES results led to the change in management was it recorded. We did not require a genetic diagnosis be achieved for there to be a change in medical management. In fact, multiple ICU physicians independently noted that negative results were also helpful in their decision making. Recommendations for cascade family testing, variant of uncertain significance (VUS) resolution testing and identifying recurrence risk for couples was also tracked and recorded, but these were not classified as management changes of the patient.

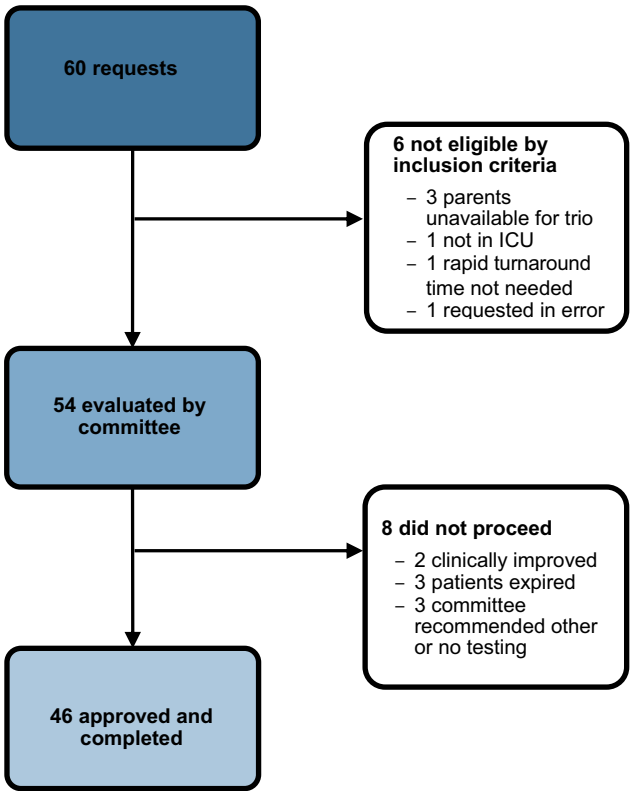

Figure 1. Case selection flow chart for RIGhT study.

Results

During the 33-month study period, 60 cases were referred to the laboratory genetic counselor by a consulting geneticist for consideration (**Figure 1**). Six cases were declined because they did not meet the inclusion criteria. Fifty-four cases were reviewed by the committee and, of those, 46 (85%) were unanimously approved for rES, 19 of which included mitochondrial DNA testing. The most common reason for exclusion was nonavailability of both parents for trio rES and 3 patients expired before sample send out. Multiple congenital anomalies with or without congenital heart defect, respiratory failure, and heart failure were the primary test indication in about one-half of the cases (24/46 [52%]). Other common test indications were hydrops, seizures, arthrogryposis, skeletal dysplasias, and metabolic abnormalities (**Figure 2**; available at www.jpeds.com).

The median patient age at the time of the consulting geneticist's request was 25 days with a range of 1 day to more than 15 years (**Table I**; available at www.jpeds.com). Patients had a median 5-day length of stay in the ICU before the consulting geneticist's request to the committee. More than one-half (56%) of the participants were in the neonatal ICU, 22% were in the pediatric ICU, and 22% were in the cardiac ICU. Nine patients (20%) were on extracorporeal membrane oxygenation (ECMO) mechanical cardiopulmonary support in the ICU.

For 21 patients (46%), rES was the first genetic test. Of the 25 patients (54%) who had previous genetic testing, 20 had a prior nondiagnostic single nucleotide polymorphism array and 7 had prior molecular testing such as a single gene or panel test. Twenty-eight patients (61%) had prior or concurrent biochemical testing. Twenty-eight cases (61%) had pathology results (biopsy or autopsy) in addition to the rES.

The median turnaround time from rES request to verbal result was 9 calendar days (range, 5-26 days). The median turnaround time from sample send out to verbal results was 6 calendar days (range, 5-10 days). Therefore, the process of committee review, pretest counseling, and sample collection from the patient and parents took about 3 calendar days. Committee review took a maximum of 1 business day. Most of this 3-day period between rES request and sample send out was taken up by pretest counseling and obtaining parental samples, which could be difficult if the parents were not present at the time of consultation. Even with rapid turnaround times, 5 patients expired before return of verbal results. In 2 of those patients, a molecular diagnosis was achieved post mortem.

A molecular diagnosis was made in nearly one-half of patients (20/46 [43%]) (**Table II**). In addition, there were 11 uncertain diagnoses and 4 partial diagnoses (**Table II**). Of the confirmed diagnoses, 13 were single nucleotide variants (SNVs), 5 were copy number variants (CNVs), 1 was a case of maternal uniparental heterodisomy, and 1 was a

Table II. Cases with a molecular diagnosis by rES

IDs/Sex/Age*	Phenotype (human phenotype ontology terms)	Disease (OMIM# if available)	Gene/Chromosome	Variant(s)	Inheritance	rES as initial test Y/N
048/F/2	Respiratory failure HP:0002878	LADD syndrome with acinar dysplasia (OMIM#149830)	*FGF10*	c.524delA, p.M176CfsX5	Autosomal dominant— paternally inherited	Y
028/F/126	Seizures HP:0001250 Abnormal movements HP:0100022	Early infantile epileptic encephalopathy 13 (OMIM# 614558)	*SCN8A*	c.2549G>A, p.R850Q	Autosomal dominant—de novo	Y
020/M/4	Respiratory failure HP:0002878	1p36 deletion syndrome (OMIM #607872)	1p36.23	(7258622_9097054) x1	Autosomal dominant—de novo	Y
058/M/419	Mitral valve stenosis HP:0001718 GDD HP:0001263	17q21 deletion	17q21	(46314762_48787388)X1	Autosomal dominant—de novo	Y
007/F/36	Hypoplastic left heart HP:0004383 Dysmorphic features HP:0000271	Koolen de Vries syndrome (OMIM #610443)	*KANSL1*	c.1579_1582delATTG p.1527VfsX50	Autosomal dominant—de novo	N, prior CMA
008/F/4	Complex congenital heart defect HP:0001627 Brain malformation HP:0012443 Dysmorphic features HP:0000271	Chromosome 2q deletion	2q14.2q23.1	(120926106_149857498)x1	Autosomal dominant—de novo	Y
012/M/1	Nonimmune Hydrops Fetalis HP:0001790	Generalized arterial calcification of infancy 1 (OMIM #208000)	*ENPP1*	c.2662C>T, p. R888W and c.913C>A, p.P305T	Autosomal recessive	Y
016/M/71	Skeletal dysplasia HP:0002652	Spondyloepiphyseal dysplasia congenita (OMIM #183900)	*COL2A1*	c.1403G>A, p.G468D	Autosomal dominant—de novo	N, prior CMA
022/F/1	Nonimmune Hydrops Fetalis HP:0001790	Noonan syndrome (OMIM #610733) and †Biotinidase deficiency (OMIM #253260)	*SOS1* and *BTD*	c.806T>C, p.M269T and c.1330G>C, p.D444H c.1368A>C, p.Q465H	Autosomal dominant—maternally inherited and Autosomal recessive	N, prior prenatal CMA
027/F/5	Hyperammonemia HP:0008281	Carbonic anhydrase 5a deficiency (OMIM #615751)	*CA5A*	c.721G>A, p.E241K homozygous	Autosomal recessive	Y
029/F/83	Respiratory insufficiency HP:0002093 Pierre-Robin sequence HP:0000201	LADD syndrome (OMIM #149730)	*FGF10*	c.577C>T, p.R193X	Autosomal dominant—de novo	N, prior CMA
030/M/1	Respiratory failure HP:0002878 Dysmorphic features HP:0000271	17q23 deletion	17q23	(59290909_61353248)x1	Autosomal dominant—de novo	Y
031/M/2	Arthrogryposis HP:0002804	Congenital myasthenia syndrome (OMIM #616314)	*CHRNB1*	Exon 8 deletion And c.1218-9_1218-7delCTC	Autosomal recessive	Y (though prior affected fetus with negative CMA)
037/F/16 years	Renal failure HP:0001919	Juvenile nephronophthisis (OMIM #256100)	2q13 (inclusive of *NPHP1*)	(110862477_110964737)x0	Autosomal recessive	N, prior single gene testing

(*continued*)

Table II. Continued

IDs/Sex/Age*	Phenotype (human phenotype ontology terms)	Disease (OMIM# if available)	Gene/Chromosome	Variant(s)	Inheritance	rES as initial test Y/N
047/F/1	Complex congenital heart defect HP:0001627 Heterotaxy HP:0030853 Congenital hydrocephalus HP:0000238	20p11 deletion	20p11	(21,680,345_24,383,453)x1	Autosomal dominant—de novo	N, CMA sent concurrently
054/F/59	Seizures HP:0001250 Jejunal atresia HP:0005235	Chromosome 15 maternal uniparental disomy	Chromosome 15	upd(15)mat	UPD	N, prior CMA
063/F/23	Nonimmune Hydrops Fetalis HP:0001790	Lymphatic malformation (OMIM #617300)	*EPHB4*	c.2288G>A, p.R763Q	Autosomal dominant—maternally inherited	N, prior CMA
064/M/146	Cardiomyopathy HP:0001638	SCN5A-related dilated cardiomyopathy (OMIM #601154)	*SCN5A*	c.5129C>T, p. S1710L and c.680T>C, p.L227P	Autosomal semidominant—maternally and paternally inherited	Y, prior CMA
068/M/5	Cardiomyopathy HP:0001638	MYH7-related cardiomyopathy (OMIM #613426)	*MYH7*	c.2292C>A, p.F764L	Autosomal dominant—paternally inherited	Y
078/M/241	Congenital diaphragmatic hernia HP:0000776 Dysmorphic features HP:0000271 Congenital hypothyroidism HP:0000851	Cutis laxa (OMIM #613177) and †congenital hypothyroidism (OMIM #274700)	*LTBP4* And *TG*	c.14T>G, p. V5G Homozygous And c.3217+5G>A and c.3999C>G, p.I1333M	Autosomal recessive and autosomal recessive	N, prior CMA
Partial diagnosis						
069/M/13 years	Cardiomyopathy HP:0001638 and intellectual disability HP:0001249	8p12 deletion	8p12	(29317218_32518884)x1	Autosomal dominant—paternally inherited	Y
009/M/163	Liver failure HP:0001399 Hemolytic anemia HP:0001878 Cardiomyopathy HP:0001638	Hereditary spherocytosis (OMIM #182900)	*ANK1*	c.353C>A, p.A118E	Autosomal dominant—paternally inherited	N, prior hemophagocytic lymphohistiocytosis panel
074/M/39	Respiratory failure HP:0002878 Hypoalbuminemia HP:0003073	Congenital analbuminemia (OMIM #616000)	*ALB*	c.412C>T, p.R138X and c.714-2A>G, IVS6-2A>G	Autosomal recessive	Y
035/F/18	Arrhythmia HP:0011675 Cardiomyopathy HP:0001638 Brain malformation HP:0012443 Dysmorphic features HP:0000271	4H leukodystrophy (OMIM #614381)	*POLR3B*	c.1939G>A, p.E637K and c.237delG, p.M794CfsX16	Autosomal recessive	N, prior CMA

LADD, lacrimo-auriculo-dento-digital; *UPD*, uniparental heterodisomy.
*Age at rES request in days unless otherwise noted.
†Denotes dual diagnosis.

compound heterozygote for an autosomal recessive condition with an SNV and an exonic deletion. Nine diagnoses were de novo dominant, 4 were inherited autosomal dominant, 5 were autosomal recessive, and 1 was semidominant. None were X-linked or mitochondrial. Diagnostic yield by primary test indication is shown in Figure 2.

Seventy percent opted to receive for secondary findings and in 4 cases a secondary result was found. These included pathogenic or likely pathogenic variants in *RET*, *PMP22*, and *BRCA2* (× 2). In these 4 cases, the primary result was either negative or uncertain.

Of all patients who underwent rES, 52% had a change in clinical management including, in some cases, those with a negative result (Table III). There were 41 total changes in management in 24 patients: 19 patients with a diagnostic test and 5 with a nondiagnostic test (Figure 3). There were 9 changes in testing, 9 changes in medication, 5 changes in imaging, and 8 changes in surgical planning. In 5 families, the results led to a change in goals of care such as limiting life-sustaining support or not escalating support. In 5 cases, the ICU physicians pursued a more aggressive course of care, such as ECMO support (case 032) or invasive diagnostic testing (case 079), because of negative rES results (Table III).

Family cascade genetic testing or other medical evaluation was recommended for 6 families. In most cases, this evaluation was an echocardiogram for parents and family members of a child with cardiomyopathy. Family cascade testing or other medical evaluation was not classified as a change in management.

Discussion

We studied trio rES in 46 children in ICUs at a single tertiary institution. We demonstrated an overall diagnostic rate of 43%. An additional 30% had an uncertain result or partial diagnosis. Two patients had dual diagnoses (case 022: Noonan syndrome and biotinidase deficiency; case 078: cutis laxa and congenital hypothyroidism). Twenty-four participants (52%) experienced a change in medical or surgical management as a direct consequence of the exome sequencing result (Figure 3 and Table III). This includes 5 patients (032, 046, 044, 043, and 079) in whom a nondiagnostic rES result led to in a change in management (Figure 3 and Table III).

Previous studies have demonstrated the clinical usefulness of rES or rapid genome sequencing.[4,6-13] However, our study is unique in that all families were provided with pretest counseling and were given the option to receive secondary findings. Our study did not perform rES in-house, but sent samples out to a laboratory (GeneDX), making it applicable to a larger number of pediatric institutions. Our study was done as a part of routine clinical care and was not subsidized by research or other funds, and our study did not consider determining recurrence risk for family planning and family cascade testing/screening as a change in management. Our study tracked VUS resolution testing. Our results represent an approach to ICU-based rapid genomic diagnostics that can be more broadly representative of clinical practice.

Given the high cost of rES and potential for errors in ordering this test, we established a committee of 4 clinical geneticists who had to review each request.[16] Although the inclusion of a review committee added an extra day to the process, the review process was standardized, and testing performed if clinically indicated. Consensus criteria for determining which neonates benefit from rapid testing have only recently emerged, and at the present most health plans in the US still do not cover this type of testing. We anticipate that results from our study and others will lead to more standardized criteria and health plan coverage changes, obviating the need for review committees and assisting with coverage for testing.

The median turnaround time from rES request to verbal result was 9 calendar days. Although this is slower than what has been reported with ultrarapid genome sequencing, it is comparable with several previous reports.[7-13,24] Previous studies of rapid sequencing have often focused on the speed of the test itself, that is, the DNA sequencing and variant interpretation.[24,25] It is important to note that our median 9-day turnaround time includes steps (pretest counseling, coordination of parental samples, and shipping to the send out laboratory) upstream of DNA extraction. Nonetheless, there are several ways our process could be sped up further. As mentioned, removing the requirement of committee review could shorten the process by 1 day. However, the biggest delaying factor was parental coordination, including sample collection, which has been demonstrated in previous studies.[13,26] For example, the mothers of several infants were not available at the time of genetics consultation because they remained at the birthing hospital. It is possible that proband-only analysis, which does not require parental samples, could improve turnaround time without significantly sacrificing diagnostic yield.[27] Turnaround time will also continue to improve with the application of more advanced automated variant interpretation algorithms.[25]

Another source of delay to diagnosis was our requirement of nondiagnostic CMA as an inclusion criterion. Partway through our study, the reference laboratory included CNV detection as a part of rES, and this inclusion criterion was removed. Subsequently, 6 patients were identified to have a CNV by rES. The smallest detected CNV was a biallelic 102-kb deletion of *NPHP1* causing juvenile nephronophthisis in case 037 (Table II). This finding supports the use of rES as a first-line test to detect both SNVs and CNVs to decrease diagnostic delay. This finding is consistent with previous studies that have suggested greater diagnostic usefulness for exome sequencing compared with CMA, particularly in hospitalized children.[27]

In several cases, the results from rES led to medical therapies that would not have otherwise been initiated (Table III). A neonate (case 031) with arthrogryposis was found to have *CHRNB1*-related congenital myasthenia

Table III. Patients who experienced changes in management

ID	Diagnosis or phenotype	Medication	Testing	Imaging	Surgical planning	Goals of care
Neonates ≤28 days						
022	Noonan syndrome and biotinidase deficiency	Biotin supplementation	1. Biotinidase activity 2. Coagulation studies	Renal ultrasound examination	–	–
047	20p11 deletion	1.Levothyroxine 2.Hydrocortisone	Monitored liver function tests and bilirubin levels	Magnetic resonance imaging of the brain	Proceeded with cardiac surgery	–
012	Generalized arterial calcification of infancy	Bisphosphonates	Additional planned diagnostic testing cancelled	–	Avoided need for arterial biopsy	–
031	Congenital myasthenia syndrome	Acetylcholinesterase inhibitors	Electromyography	–	–	–
008	2q-	–	–	–	Planned surgical intervention for free air in the abdomen was cancelled due to poor neurologic prognosis	Limited life sustaining care
020	1p36 deletion syndrome	–	1. Thyroid-stimulation hormone 2. Audiology evaluation	Renal ultrasound examination	–	–
068	*MYH7*-related cardiomyopathy	–	–	–	Proceeded with heart transplant	–
027	Carbonic anhydrase VA deficiency	Resumed a non-protein-restricted diet and discharged with a no-protein sick day plan.	–	–	–	–
035	Possible 4H leukodystrophy	–	–	–	Family declined heart transplant	Limited life-sustaining care
063	*EPHB4*-related lymphatic dysplasia	Sirolimus	–	–	–	–
032	Cardiomyopathy	–	–	–	–	Escalated care—started on ECMO while awaiting transplantation
046	Respiratory failure	–	–	–	–	Escalated care—maintained continuous renal replacement therapy
Infants 1 month-1 year						
074	Congenital analbuminemia	Increased albumin and weaned diuretics	–	–	–	–
007	*KANSL1*-related disorder	–	–	Hip ultrasound examination	Family opted to proceed with G-tube placement given diagnosis associated with feeding difficulties	–

(*continued*)

Table III. Continued

ID	Diagnosis or phenotype	Medication	Testing	Imaging	Surgical planning	Goals of care
016	Spondyloepiphyseal dysplasia congenita	–	–	Cranial ultrasound examination to evaluate for hydrocephalus	–	–
054	upd(15)mat	–	Endocrine labs to evaluate for adrenal insufficiency	–	–	–
029	LADD syndrome	–	–	–	Parents opted for tracheostomy and gastrostomy feeding tube placement	–
028	*SCN8A*-related early infantile epileptic encephalopathy	Antiepileptic drugs transitioned to sodium channel blocker	–	–	Given poor prognosis for recovery and high risk for sudden death from epilepsy (SUDEP), family opted for tracheostomy	Goal changed from cure to going with home ventilator
078	*LTBP4*-related cutis laxa and congenital hypothyroidism	–	–	–	–	Family agree to no further escalations of care
044	Seizures	–	–	–	–	Escalated care—tracheostomy
043	Postnatal hydrops	–	–	–	–	Escalated care—continued diuretic therapy for anasarca
079	Severe ichthyosis	–	–	–	–	Escalated care—invasive testing, including bone marrow and renal biopsies
Children >1 year						
058	17q21 deletion	–	–	–	–	Limited life-sustaining care
037	Juvenile nephronophthisis	–	Ophthalmologic evaluation	–	–	–

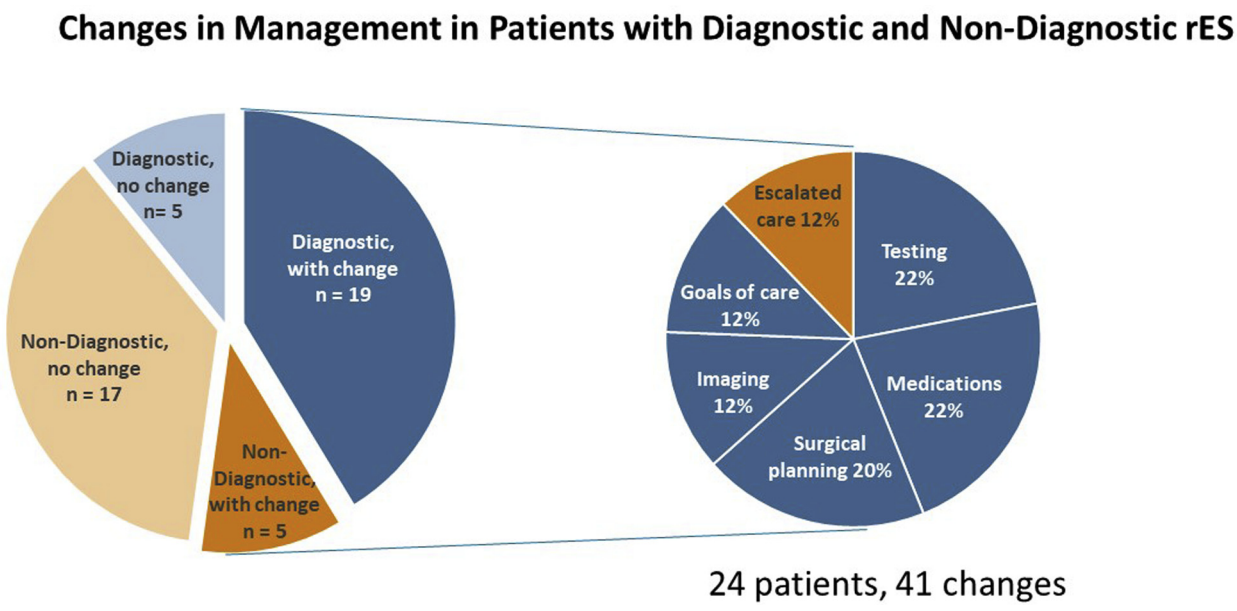

Figure 3. In 19 of 24 patients with a diagnostic rES, there was at least 1 change of clinical management, although most had more than 1 change. There were 41 changes in 5 categories displayed (testing, medications, surgical planning, imaging, and goals of care, percent given as a percentage of total changes). Patients with nondiagnostic exomes also had changes of management. Of 22 patients with a nondiagnostic exome, 5 had escalations of care after return of results (12 percent of total changes).

syndrome. After confirming pyridostigmine-responsive myasthenia on electromyography, the patient was started on acetylcholinesterase inhibitors, which permitted extubation. A hydropic infant (case 012), with possible generalized arterial calcification of infancy based on imaging, was able to be placed on bisphosphonates after this diagnosis was molecularly confirmed. A 4-month-old with seizures and abnormal movements (case 028) was changed to a sodium channel blocking antiepileptic medication after *SCN8A*-related epilepsy was identified. Sirolimus was started in a neonate with hydrops (case 063) after *EPHB4*-related primary lymphatic dysplasia was diagnosed.

Primary respiratory failure in neonates was a common indication for rES and highlights the importance of coordination with pathology, as traditionally infants with severe interstitial lung disease have been diagnosed via invasive lung biopsy.[28] In case 030, rES was sent to avoid lung biopsy while on ECMO (**Table II**). However, the patient clinically worsened and a lung biopsy provided a diagnosis of acinar dysplasia before rES results. Life-sustaining support was discontinued; thus, case 030 represents an example of a diagnostic result that did not change patient management. Nonetheless, the identification of a de novo 17q23 deletion as the cause of this child's lung disease, even if reported posthumously, provided closure and assistance in family planning.[29] In the future, rapid genome sequencing, which has the potential for even faster turnaround times, will likely replace rES as the primary diagnostic test. Indeed several publications have already shown the clinical usefulness of rapid genome sequencing, although this test is not yet broadly clinically available.[4-15]

In 4 cases, an inherited dominant diagnosis was made. In one of those cases, a neonate with severe cardiomyopathy (**Table II**, case 068), the father was identified to be mosaic for a pathogenic variant by exome sequencing. He was asymptomatic but an echocardiogram was recommended. In 2 cases—022 and 063 (**Table II**)—the parent was diagnosed for the first time. In case 022, the infant was prenatally identified to have hydrops and found to have Noonan syndrome. The patient's mother had a history of pulmonic stenosis requiring repair in her 20s, but was otherwise well and had not previously been diagnosed with Noonan syndrome. In case 063, a maternal family history of lymphedema was identified but the cause was not known. Last, case 048 presented with severe respiratory failure and was found to have lacrimo-auriculo-dento-digital syndrome. Her father and other paternal family members had had lacrimal duct stenosis as children, but were otherwise healthy.

A major impact of rES results on patient care was guiding goals of care (**Table III**). We found that both diagnostic and nondiagnostic rES could change goals of care in both directions, toward more and less aggressive care. For example, a decision to limit aggressive medical intervention was made for a neonate with multiple congenital anomalies (case 008) after rES identified a genetic diagnosis consistent with poor neurologic prognosis (chromosome 2q deletion). In contrast, continued aggressive care was chosen for an infant (case 016) with a severe skeletal dysplasia whose

rES results (*COL2A1*-related spondyloepiphyseal dysplasia) predicted a normal neurologic outcome. A nondiagnostic rES result led to the decision to begin ECMO support in an infant with hydrops and cardiomyopathy (case 032), because the result decreased the probability of a syndromic diagnosis.

Previous reports of rapid genomic testing, many of which have taken place in the research setting, have primarily reported only diagnostic or nondiagnostic results.[4,6-15] This finding is not representative of standard clinical genetic test results, which will include VUSs and other inconclusive results. Additional follow-up testing to further classify VUSs is commonly required in standard clinical genetic practice. In this study, we tracked both the number of inconclusive results and the health care burden associated with those. Ten patients (22%) had additional actions owing to inconclusive results, including 13 additional laboratory or imaging tests. Two patients were enrolled in research studies to further investigate a VUS. We did not include any of this testing as a change in management, but it is important that future studies examining the cost effectiveness of rES track the burden associated with follow-up testing. Although this study does not address the cost effectiveness of rES in the ICU, previous studies have demonstrated the potential for rapid genetic diagnosis to significantly decrease inpatient costs.[4,17]

Because all testing was done as trios, we were able to distinguish between inherited and de novo variants. For 11 trios, the pathogenic variant(s) were inherited, conferring a significant recurrence risk for subsequent pregnancies. We chose not to include changes in reproductive risk counseling as a "change in management" in this study. This information, although extremely valuable to families, does not change inpatient management. Additionally, insurance companies do not generally reimburse genetic testing solely for the purpose of family planning. Nonetheless, information regarding recurrence risk counseling is routinely obtained from diagnostic rES and can provide an added benefit to families of children who are critically ill.

Few previous studies of rapid genomic testing within ICUs have reported secondary findings.[4,6-13] In a previous study, 7 of 267 participants had medically actionable secondary findings.[9] In our study, 70% of patients opted to receive secondary findings. Four patients (9%) had secondary findings inherited from a parent who also received the results. In all 4 patients, the rES was nondiagnostic for the primary test indication, which led to challenging post-test counseling discussions.[26]

Our study provides evidence that trio rES for critically ill children with a suspected monogenic disorder has had a significant impact on medical care within our patient population. We have demonstrated that rES has led to a change in management in more than 50% of patients tested. Because this test is able to detect both CNVs and SNVs, rES should be considered a first tier test in critically ill children with suspected genetic disease. We believe this test should become standard of care for tertiary institutions that can provide coordinated genetics expertise. ■

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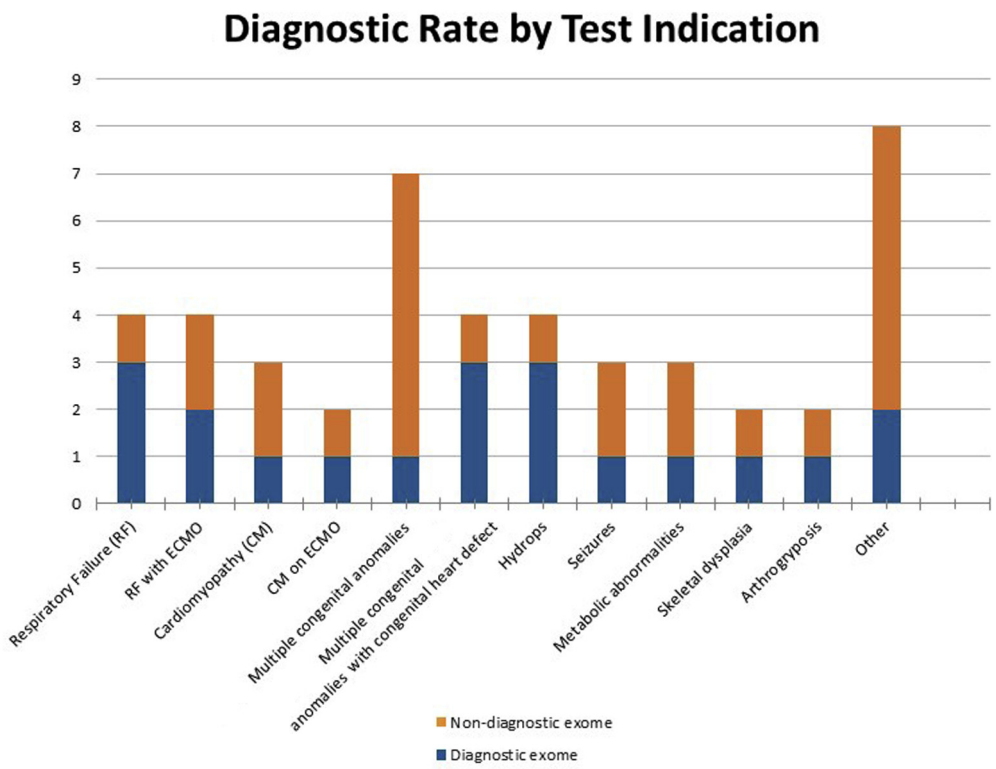

Figure 2. The number of diagnostic and nondiagnostic exomes by test indication. The most common category was multiple congenital anomaly both with and without congenital heart defect. Other includes all indications which only appeared once in our data such as liver failure and renal failure.

Table I. Patient characteristics and prior testing (n = 46)

Median age at request (min, mean, max)	25 days (1 days, 297 days, 15 years)
Median days in ICU before genetics consult (min, mean, max)	5 days (0, 21, 241)
Type of ICU	Neonatal 26 (56%) Pediatric 10 (22%) Cardiac 10 (22%)
On ECMO	9 (20%)
rES as initial test	21 (46%)
rES as second tier test	25 (54%) - Prior CMA: 20 - Prior single gene or panel test: 7