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Targeted gene panel sequencing for the rapid diagnosis of acutely ill infants

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Abstract

Background: Exome/genome sequencing (ES/GS) have been recently used in neonatal and pediatric/cardiac intensive care units (NICU and PICU/CICU) to diagnose and care for acutely ill infants, but the effectiveness of targeted gene panels for these purposes remains unknown.

Methods: RapSeq, a newly developed panel targeting 4,503 disease-causing genes, was employed on selected patients in our NICU/PICU/CICU. Twenty trios were sequenced from October 2015 to March 2017. We assessed diagnostic yield, turnaround times, and clinical consequences.

Results: A diagnosis was made in 10/20 neonates (50%); eight had de novo variants (*ASXL1*, *CHD*, *FBN1*, *KMT2D*, *FANCB*, *FLNA*, *PAX3*), one was a compound heterozygote for *CHAT*, and one had a maternally inherited *GNAS* variant. Preliminary reports were generated by 9.6 days (mean); final reports after Sanger sequencing at 16.3 days (mean). In all positive infants, the diagnosis changed management. In a case with congenital myasthenia, diagnosis and treatment occurred at 17 days versus 7 months in a historical control.

Conclusions: This study shows that a gene panel that includes the majority of known disease-causing genes can rapidly identify a diagnosis in a large number of tested infants. Due to simpler deployment and interpretation and lower costs, this approach might represent an alternative to ES/GS in the NICU/PICU/CICU.

KEYWORDS

genetic diagnosis, neonatology, newborn, precision medicine, rapid sequencing

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1 | INTRODUCTION

The ability to decipher the genetic code of individual patients has markedly improved over the last 10 years due to exponentially decreasing costs and improved accuracy of next-generation sequencing (NGS) as well as rapidly growing libraries of known genetic conditions with associated sequences (Ng et al., 2010; Taylor et al., 2015; Worthey et al., 2011). NGS assays include sequencing a targeted panel of genes, exome sequencing (ES), which involves sequencing the coding and the intron-exon boundary regions of almost all human genes, and genome sequencing (GS). Targeted gene panels have lower cost than ES or GS and provide excellent coverage depth, but some genes of interest may not be included. ES has lower cost than GS, but the coverage of some exons might be suboptimal, especially in GC-rich regions (Belkadi et al., 2015; Meienberg, Bruggmann, Oexle, & Matyas, 2016). GS provides the most comprehensive coverage, but is also the most expensive (Belkadi et al., 2015).

Exome and genome sequencing with a typical turn-around time of weeks to months have revolutionized the diagnosis and treatment of diseases such as developmental disorders and cancer (Deciphering Developmental Disorders, 2017; Hyman, Taylor, & Baselga, 2017). The application of these tests in neonatal and pediatric/cardiac intensive care units (NICUs, PICUs/CICUs) has remarkable appeal. The recent development of rapid ES and GS (rES and rGS) with turn-around-time of 1–2 weeks has allowed the successful application of these tests in NICUs and PICUs/CICUs, identifying unexpected numbers of acutely ill infants with single-gene disorders, and positively changing their care (Daoud et al., 2016; Meng et al., 2017; Saunders et al., 2012; Soden et al., 2014; van Diemen et al., 2017; Willig et al., 2015). Despite these successes, rES and rGS remain costly and are not readily available to the majority of intensive care units caring for infants and children.

Examining a panel of genes with known function and established disease associations would simplify data interpretation. Thus, we hypothesized that a rapid targeted gene panel might provide a simpler alternative to rES and rGS, yet with comparable diagnostic rates. After developing a rapid, customized gene panel of 4,500+ disease-causing genes (RapSeq), we tested its application in our NICU and PICU/CICU (Brunelli et al., 2017). Our results support the utility of deploying rapid targeted gene panels in these settings, suggesting that additional studies are needed to define their relative performance and value compared to rES and rGS.

2 | METHODS

2.1 | Ethical compliance

The analysis and publication of data related to this clinical program were approved by the Institutional Review Board at the University of Utah with a waiver of consent and authorization.

2.2 | Specimens

Twenty trios (newborns and their biological parents) were tested from October 2015 to March 2017. Families were offered the option of RapSeq based on the acuity/disease severity (Table 1). Pretest genetic consultation and parental consent were conducted by neonatologists, a neonatal neurologist, geneticists, genetic counselors, and

TABLE 1 RapSeq selection criteria

1) Meets our definition of "high acuity"	Patients present with respiratory and/or cardiovascular failure, encephalopathy, profound hypotonia, complex brain malformations, severe metabolic disturbance, or multiple congenital anomalies (without a known syndrome), unusually severe or prolonged disease, or multi-system organ failure.
2) Making a diagnosis will alter acute decision-making	Consensus that time-critical treatment and/or change in care plan might be anticipated based on the results. Examples include administration of medications and diagnostic testing that could confirm a diagnosis. Importantly, irreversible decisions such as withdrawal of care are *not* based solely on genetic testing, especially in case of preliminary results.
3) The patient's disease is plausibly monogenic	The presentation is within a spectrum with likely genetic causes, but for which a clear diagnosis cannot be made. Examples of conditions where this would not be the case include isolated congenital diaphragmatic hernia or isolated congenital heart disease.
4) Alternate testing is unavailable, more costly, or protracted	Examples where alternative testing is more appropriate include chromosomes for suspected trisomy and single gene testing or multigene panels for clear-cut genetic conditions (unless turn-around-time is excessive). Tests that might best be done in parallel include chromosome microarray, SMA testing, MRI, and metabolic testing. Tests that might best be done in series include muscle biopsy, CSF neurotransmitters.
5) A trio (*both* parents and patient) is available.	For a rapid interpretation, only trios are tested because this speeds the filtering process, allows identification of de novo variants, and establishes phase of potentially recessive alleles without the delays due to Sanger validation.

trained nurses. Informed consent for the clinical test was obtained in all cases and documented the following risks: the chance that the cause of the medical condition would not be determined, the possibility of future employment or insurance discrimination, the discovery of nonpaternity, learning the infant's diagnosis was not treatable, and determination that another family member is a carrier of/or affected with the same condition. One of the points discussed in the consent form signed by parents was related to the Genetic Information Nondiscrimination Act (GINA), and the consent form included a link to the National Human Genome Research Institute website that provides additional information about GINA (http://www.genome.gov/10002328). Information provided on the consent indicated that a preliminary result would typically be available in 7–10 days and the final result in 14–21 days. It also stated that only variants related to the infant's diagnosis would be reported. Parents also indicated whether they wanted to be informed of secondary findings related to a pathogenic variant identified in one of the 59 recommended American College of Medical Genetics genes (Richards et al., 2015). A minimum sample of 1 ml peripheral blood was collected in EDTA (lavender top tube) from the proband and the biological parents and immediately sent to the laboratory along with a copy of the specific Consent Form signed by a legal guardian. Additionally, a patient history form was completed and submitted with the specimen detailing all clinical findings, results of previous tests such as newborn screening, chromosomal microarray analysis (CMA), imaging studies, and a three-generation family pedigree. Upon arrival in the laboratory, the paperwork was immediately reviewed by a genetic counselor to confirm receipt of necessary samples and completion of the required consent and paperwork; the genomic laboratory was then alerted to the presence of the specimens for immediate testing.

2.3 | A rapid targeted gene panel with 4,500+ known disease-causing genes

We used a human, nuclear, inherited disease panel with biotinylated DNA probes designed to target 4,503 disease-causing genes (Brunelli et al., 2017). Genomic DNA was isolated from whole blood and sonicated to obtain double stranded DNA fragments between 250 and 300 base pairs in length. The sonicated genomic DNA was processed to create NGS whole genome libraries that were subsequently hybridized to the biotinylated DNA probes to capture genomic regions of interest. After hybridization, unwanted genomic DNA was removed through washing, and the final captured libraries were amplified by PCR. A single capture was used to create the proband's genomic library whereas the parents' NGS libraries were pooled and a single capture was used prior to sequencing on the Illumina NextSeq 500. After sequencing, the Fastq files were processed with an internal bioinformatics pipeline for alignment and variant calling using BWA, Samtools, GATK, FreeBayes, SNPeff, and proprietary software for alignment and variant calls. The probands' variants were filtered to search for any variants potentially associated with reported clinical phenotypes.

2.4 | Results interpretation and reporting

Since RapSeq was developed to determine the cause of medical findings in acutely ill newborns believed to have a genetic condition, data analysis and interpretation focused on pathogenic variants or variants of unknown significance in genes potentially causative for the patient's clinical phenotype. Given the rarity of most genetic diseases, variants with an allele frequency of >1% in the Broad Institute Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/) and gnomAD (https://gnomad.broadinstitute.org/) browsers were filtered out. The proband's data were analyzed for de novo coding and splice region variants by filtering out the variants present in either parent. All variants were analyzed under inheritance patterns that included X-linked, autosomal dominant (AD) inheritance with variable expressivity/penetrance, and autosomal recessive (AR) inheritance which included looking for single variants since one of the variants may not be detected by NGS methodology (e.g., insertion/deletion). Pathogenic variants were confirmed with Sanger sequencing.

3 | RESULTS

Twenty infants (30% female, Table 2) underwent RapidSeq. At the time of blood sample collection, the age of probands ranged from 1 day to 5 months. Although our enrollment criteria specified the need for the availability of both parents' samples, one infant with only one available parent was enrolled in an attempt to clarify a particularly severe clinical course. A preliminary report was issued in 6–15 days (mean 9.6 days) with an overall summary result of either "positive" or "negative". Positive results provided the putative gene but not the specific variant(s). Final reports were issued in 8–24 days (mean 16.3 days) following Sanger sequencing confirmation of the variant(s) included in the final report. All final reports provided confirmation of the preliminary findings.

Causative variants were identified in 10 cases (Table 2). In one additional case, the phenotype could not be fully

TABLE 2 Characteristics of tested patients and results of RapSeq

Proband ID	Clinical indication	Causal gene	Disease	Inheritance pattern	Causal variants (GRCh37)	Atypical Presentation or Incomplete Diagnosis	Other genetic testing	Age at time of testing (days)
1	Symmetric IUGR, microcephaly, DORV, VSD, arthrogryposis	ASXL1 (NM_015338.5)	Bohring-Opitz syndrome (MIM:605039)	AD, de novo	chr20:g.31021270dupT; c.1269dupT; p.Leu424fs	Yes (Double outlet Right Ventricle)	Microarray: deletion of 500 kb on 13q31.3	44
2	Respiratory failure, absent cranial nerve reflexes, hypotonia, multiple joint contractures	CHAT (NM_020549.4)	Congenital presynaptic myasthenic syndrome 6 (MIM:254210)	AR, variants in trans	chr10:g.50827789G>A; c.406G>A; p.Val136Met /g.chr10:50863169G>A; c.1663G>A; p.Glu555Lys	No	None	17
3	Respiratory failure, TEF, AV canal, cortical dysplasia with polymicrogyria, cerebellar dysgenesis, choanal atresia	CHD7 (NM_017780.3)	CHARGE syndrome (MIM:214800)	AD, de novo	chr8:g.61713053_61713073delinsAGTAC; c.2345_2365delinsAGTAC; p.Ser782Ter	Yes (polymicrogyria)	Normal FISH	38
4	Respiratory failure, TAPVR, absent ear canals, nonpatent urethra, imperforate anus, limb defects, ventriculomegaly	FANCB (NM_001018113.1)	Fanconi anemia, complementation group B/ /VACTERL-H (MIM:300514)	X-linked, de novo	chrX:g.14883307del; c.326delT; p.Leu109fs	No	Normal Microarray & Chromosomes	15
5	Respiratory failure, cardiac poly-valvular dysplasia, multiple joint contractures	FBN1 (NM_000138.4)	Marfan syndrome (MIM:154700)	AD, de novo	chr15:g.48780430C>T; c.3217G>A; p.Glu1073Lys	No	Microarray: low level mosaicism for X chromosome monosomy	18
6	Respiratory failure, periventricular gray matter heterotopia, ventriculomegaly, coarctation of the aorta, ASD, VSD	FLNA (NM_001456.3)	Periventricular heterotopia (MIM:300049)	X-linked, de novo	chrX:g.153599243A>T; c.371T>A; p.Ile124Asn	No	Normal Microarray	55
7	Respiratory failure, hypocalcemia, hypothyroidism	GNAS (NM_000516.4	Pseudohypoparathyroidism type 1a/Albright's hereditary osteodystrophy (MIM:103580)	AD, maternally inherited	chr20:g.57480498C>T; c.493C>T; p.Arg165Cys	Yes (incomplete diagnosis)	Normal Microarray	31
8	Biliary atresia, microcephaly, hypotonia	KMT2D (NM_003482.3)	Kabuki syndrome 1(MIM:147920)	AD, de novo	chr12:g.49436088C>A; c.5893G>T; p.Glu1965Ter	No	Normal Microarray	85

(Continues)

TABLE 2 (Continued)

Proband ID	Clinical indication	Causal gene	Disease	Inheritance pattern	Causal variants (GRCh37)	Atypical Presentation or Incomplete Diagnosis	Other genetic testing	Age at time of testing (days)
9	HPLH, cleft palate, hepatic cyst	KMT2D (NM_003482.3)	Kabuki syndrome 1(MIM:147920)	AD, de novo	chr12:g.49435007dup; c.6546dupC; p.Tyr2183fs	No	Normal prenatal microarray and chromosomes	26
10	Cleft palate, absent corpus callosum and olfactory bulbs, tracts and sulci	PAX3 (NM_181457.3)	Waardenburg syndrome type 1 (MIM:193500), Waardenburg syndrome type 3 (MIM:148,820) Craniofacial-deafness-hand syndrome (MIM:122880)	AD and AR, both variants de novo	chr2:g.223084907del; c.1125del; p.Ser377fs/g.223086110; c.793-4A>G	Yes	Normal microarray	34
11	Pulmonary atresia, hypoplastic right ventricle, PDA, cholestasis, butterfly vertebrae	Not identified					Normal microarray	61
12	IUGR, brain and cardiac abnormalities, cleft lip and palate	Not identified					t(5;18) (p15;p11.2), normal microarray	32
13	Tetralogy of Fallot, cleft lip/palate and duodenal atresia	Not identified					Normal microarray	21
14	Nonimmune fetal hydrops, respiratory failure, atrial septal defect, hypotonia	Not identified					Normal microarray	35
15	Hepatosplenomegaly, enlarged kidneys, anterior cleft on thoracolumbar vertebral bodies, hypotonia	Not identified					No	342
16	IUGR, coagulopathy, dysphagia, feeding difficulties, and hypertonia	Not identified					Microarray: 25 kb deletion including three exons of the AHI1 gene	68

(Continues)

TABLE 2 (Continued)

Proband ID	Clinical indication	Causal gene	Disease	Inheritance pattern	Causal variants (GRCh37)	Atypical Presentation or Incomplete Diagnosis	Other genetic testing	Age at time of testing (days)
17	Sudden apneic episode requiring CPR	Not identified					No	34
18	Premature rupture of membranes, hypotonia and cranial nerve dysfunction	Not identified					Normal microarray	40
19	Acute liver failure and cholestasis	Not identified					No	171
20	Persistent unexplained respiratory distress and gastroesophageal reflux	Not identified					No	74

explained by the pathogenic variant identified in the proband, and this was considered a partial diagnosis. Nine out of 10 cases with positive findings had previous genetic workup, including normal CMA (hg19 build) tests ($n = 6$), normal FISH ($n = 1$), CMA with a 500 kb deletion ($n = 1$), and CMA reporting low level mosaicism of X chromosome monosomy ($n = 1$) (Table 2). Of the 10 certain pathogenic findings, eight (80%) were de novo variants, one was a compound heterozygous variant (AR), and one was maternally inherited (AD) (Table 2). Among the eight de novo variants, five were AD, two were X-linked, and in one the pattern of inheritance was unclear. The five de novo AD inheritance patterns included pathogenic variants in the additional sex combs like 1, transcriptional regulator gene (*ASXL1*, Bohring-Opitz syndrome) presenting with symmetric IUGR, microcephaly, DORV, VSD, and arthrogryposis; the chromodomain helicase DNA binding protein 7 gene (*CHD7*, CHARGE syndrome) presenting with respiratory failure, TEF/EA, AV canal, cortical dysplasia with polymicrogyria, cerebellar dysgenesis, and choanal atresia; the fibrillin 1 gene (*FBN1*, Marfan syndrome) presenting with cardiac and respiratory failure, cardiac poly-valvular dysplasia, and multiple joint contractures; and the lysine methyltransferase 2D gene (*KMT2D*, Kabuki syndrome) presenting in one patient with biliary atresia, microcephaly, hypotonia, laryngomalacia, paralyzed left vocal cord, supraventricular tachycardia, and renal hypoplasia, and in another patient with hypoplastic left heart syndrome, single umbilical artery, micrognathia, low set ears, and cleft palate. The two X-linked inheritance patterns included pathogenic variants in the Fanconi anemia complementation group B gene (*FANCB*, VACTERL with hydrocephalus syndrome) presenting with respiratory failure, TAPVR, absent ear canals, nonpatent urethra, imperforate anus, limb defects, and ventriculomegaly; and in the filamin A gene (*FLNA*, periventricular nodular heterotopia) presenting with respiratory failure, hypotonia, periventricular gray matter heterotopia, ventriculomegaly, coarctation of the aorta, ASD, and VSD. The patient with unclear pattern of inheritance displayed variants in the paired box 3 gene (*PAX3*, Waardenburg syndrome) presenting with cleft palate, micro-retrognathia, abnormal ears, widely spaced nipples, hypotonia, absent corpus callosum and olfactory bulbs and tracts. The compound heterozygous variant (AR) was in the choline O-acetyltransferase gene (*CHAT*, congenital presynaptic myasthenic syndrome 6 [CMS6]) presenting with respiratory failure, absent cranial nerve reflexes, hypotonia, and multiple joint contractures. The variant that was maternally inherited (AD) was in the GNAS complex locus gene (*GNAS*, pseudohypoparathyroidism type 1a/Albright's hereditary osteodystrophy) presenting with respiratory failure and multiple endocrine resistance (hypocalcemia and hypothyroidism). All cases are discussed in detail in the Data S1.

3.1 | Effects of RapSeq on patient management

All of the 11 infants with causative variants or a partial diagnosis benefited from early and precise diagnoses by having specific interventions and/or recommendations (see Table 2 for patient numbering). These changes in clinical management were derived from the medical record documentation. These included initiation of new medications ($n = 2$, patient 2, 7), early tracheostomy and/or gastrostomy tube placement ($n = 3$, patient 1, 2, 6), immunology or endocrinology follow-up ($n = 3$, patient 7, 8, 9), platelet function tests ($n = 1$, patient 6), close monitoring for cancer ($n = 1$, patient 1), breast cancer screening in family ($n = 1$, patient 11), screening for ureteral obstruction ($n = 1$, patient 6), hearing screens and/or eye exams ($n = 3$, patient 3, 6, 10), need for specific diagnostics in future pregnancies ($n = 1$, patient 2), reassurance of no increased risk in future pregnancies ($n = 2$, patient 3, 4), monitoring for Hirschsprung disease ($n = 1$, patient 10), advice against cardiac transplant ($n = 1$, patient 5), identification of disease in a parent and relatives ($n = 1$, patient 7) or prognostic data leading to withdrawal of care ($n = 2$, patient 4, 5). Many of the positive diagnoses (6/10), were not considered part of the differential diagnosis prior to testing.

3.2 | Estimation of clinical utility and cost benefit

We compared the clinical course and cost of medical services in two patients with similar neuromuscular phenotypes: a patient who underwent RapSeq (the case, patient 2 in Table 2) and one who presented prior to the availability of RapSeq (historic control). Costs for both the facility and the physician portions were standardized by assigning local Medicaid allowed-amounts and totaled across inpatient stays. The physician allowed-amounts were calculated using the current procedural terminology (CPT) codes for all sub-specialists involved in care and the State of Utah Medicaid allowed fee schedule. The facility allowed-amounts were calculated by the State of Utah fee schedule Excel worksheet based on Medicare severity-diagnosis related groups (MS-DRGs). Both the control and the case had the same MS-DRG for their facility stay.

The historic control presented to our NICU about 1 year prior to the availability of RapSeq as a 37 5/7-week infant with hypotonia, arthrogryposis, dysmorphic features, and respiratory failure. Significant clinical history included a sibling who died at 6 months with mild myopathy and respiratory issues, and who tested negative for myotonic dystrophy, normal CMA, normal EMG nerve conductions, and MRI of the brain that showed evidence of hypoxic injury. During hospitalization, diagnostic tests and therapy included a muscle biopsy, video EEG, head ultrasound, ES, and vital stimulation therapy. The hospital course was complicated by necrotizing enterocolitis. The patient had a tracheostomy and gastrostomy tube with Nissen, and was discharged to a long term care facility at 116 days of age. At 7 months of age, ES results revealed a pathogenic variant in the cholinergic receptor nicotinic delta subunit gene (*CHRND*), which leads to a congenital myasthenic syndrome. The patient was only then started on pyridostigmine.

In comparison, Patient 2 (Table 2) was a 5-day-old full term male infant delivered after a normal pregnancy. This was the first pregnancy of young, healthy parents. In addition to respiratory failure, the infant presented with peripheral nerve disease, elevated CK, absent cranial nerve reflexes, mildly dysmorphic features, and multiple joint contractures. A brain MRI showed no structural abnormalities and normal spectroscopy. CMA and spinal muscular atrophy testing were negative. RapSeq preliminary result was delivered within 11 days of testing and showed compound heterozygous variants in the choline O-acetyltransferase gene (*CHAT*), consistent with the c.406G>A (p.Val136Met) and the c.1663G>A (p.Glu555Lys) alleles. Compound heterozygous or homozygous pathogenic variants in *CHAT* cause CMS6 (OMIM 254210). A positive neostigmine test performed within 48 hr of the preliminary report confirmed the diagnosis within the first two weeks of life, and the patient was continued on pyridostigmine. Due to the AR inheritance pattern of the disease, parents were counseled regarding recurrence risk and options for testing in future pregnancies. The parents opted for tracheostomy and gastrostomy, and the infant was discharged to an extended care facility at 65 days of age after appropriate titration of therapy. Analysis of costs demonstrated that during the level IV NICU stay our historic control patient incurred costs that were at least $71,393 higher than this

TABLE 3 Economic cost comparison of two cases by diagnostic charges and coded procedures, fee schedule for physician portions, facility cost, and overall total estimated Medicaid Allowance

	# of diagnostic charges/coded procedures	Physician allowed amount	Facility allowed amount	Total Medicaid allowed amount
Control patient	124	$31,069	$184,448	$215,517
RapSeq patient	151	$30,303	$113,822	$144,125

patient. This difference arose primarily out of facility costs (Table 3).

4 | DISCUSSION

Our rapid, large panel gene test provided meaningful information that changed the ongoing management of newborns in the NICU and PICU/CICU. Although some diagnoses did not lend themselves to effective treatments, they provided an end to what could have been an otherwise extensive diagnostic odyssey. The results also provided information regarding prognosis, accurate recurrent risk and guidance with disease management, as well as long-term intervention that could be very helpful for pediatricians caring for these infants after discharge.

Of the estimated 8,000 known genetic diseases (Amberger, Bocchini, Schiettecatte, Scott, & Hamosh, 2015; Amberger, Bocchini, Scott, & Hamosh, 2009), the majority (50%–75%) predominantly affect children. Collectively, they are the leading cause of infant mortality (about 35%) and are a leading cause of pediatric hospital admissions (Dodge et al., 2011; Yoon et al., 1997). Precise early diagnosis, that is, identifying the molecular cause (genotype) of the clinical features (phenotype), is critical to provide better care for affected infants in the immediate newborn period and after discharge as well as for accurate counseling of families. Thus, we postulate that strategies for rapid genetic diagnosis are important for the care of acutely ill infants and children.

This study demonstrates that the cost of rapid sequencing in a population of critically ill infants using RapSeq compares favorably to rGS. The cost of the RapSeq per trio was $6,000 while the cost for rGS per trio was reported to be $17,579 by Mestek-Boukhibar et al. (2018), and approximately $16,074 by Farnaes et al. (2018). The clinical sensitivity of RapSeq was also comparable if not superior to several recent rGS studies. RapSeq was able to determine a full explanation for 50% of patients' symptoms and at least a partial explanation in 55%. This compared favorably to the clinical sensitivity of rGS in NICU cohorts. Farnaes et al. reported 38% of patients received a full explanation for their symptoms by rGS while 43% received at least a partial explanation (Farnaes et al., 2018). Additionally Petrikin et al. reported a clinical sensitivity of 31% by rGS (Petrikin et al., 2018). The reason for the favorable sensitivity for RapSeq may be due to the fact that all genes known to be causative of Mendelian disorders were targeted by the panel. The panel undergoes periodic updates to add recently identified disease-causing genes. The current iteration of the panel has over 4,900 genes of known Mendelian function (up from the original 4,503 genes). Another reason for the high clinical sensitivity of RapSeq may be because all samples were submitted as a trio with the exception of one patient for which a paternal sample was not available. While Farnaes et al. also intended to only offer rGS for trios, their study accepted 30 trios, nine duos and three singletons (Farnaes et al., 2018). This increased number of duos and singletons likely decreased the clinical sensitivity as it is known that de novo variants are the most common disease mechanism in individuals who receive a diagnosis. De novo variants are much more difficult to detect in the absence of both parental samples. Also one is not able to determine if two variants in a single gene are located on opposite alleles when samples from both parents are not available.

In the current study, final reports were issued in a mean time 16 days whereas final reports required 14 and 23 days on average by rGS as reported by Farnaes et al. (2018) and Petrikin et al. (2018), respectively. RapSeq and the two studies referenced above all required confirmation of the NGS results by Sanger sequencing; this was estimated to add 7–16 days to the time of final reports. So overall, the clinical sensitivity and time to final reports for RapSeq was comparable to rGS. Our study as well as the studies by Farnaes et al. (2018) and Mestek-Boukhibar et al. (2018) thoroughly reviewed patient selection criteria for rapid sequencing Given that RapSeq is approximately three times less costly than rGS, it makes financial sense to use such a targeted panel when there is a need for a rapid and cost-effective diagnosis in a critically ill newborn.

In our cost analysis, we identified a decrease in the number of invasive procedures and diagnostic tests performed in Patient 2 compared to a historic control, leading to a shorter length of stay. Given the concerns of families and care providers for out-of-pocket costs for the care of infants in the NICU, we considered whether reductions in total costs due to the use of RapSeq testing would be passed along to the families of these patients. However, a detailed examination of local billing practices at our hospital and insurance coverages suggest that in both cases the billing totals would far exceed the families' copays and deductibles, and the remaining charges/costs would be covered 100% by insurance (whether Medicaid or private insurer). Thus, in our scenario, it is unlikely that there would be any difference in out-of-pocket costs for the families of these two patients, but there may very well be advantages for Medicaid or private insurers in limiting healthcare costs.

This study presents some limitations. In the initial 12 patients we tested (Brunelli et al., 2017), there was only one family to whom enrollment was offered but declined (enrollment rate > 90%). However, in the following patients we did not track all patients to whom testing was offered, and we are, therefore, unable to determine an overall enrollment rate. Due to limitations of RapSeq methodology, copy number aberrations could not be detected. ARUP Laboratories

is currently in the process of rigorous validation of a new in-house developed algorithm to accurately identify multi-exon CNVs, and the process will be implemented as soon as all the requirements for clinical testing in CLIA and ISO 15189-certified lab are fulfilled. Based on the published data, detection of CNVs may increase the overall detection yield of exome/medical exome sequencing cases by an additional 1.67%–16.7% (Jiao et al., 2019; Marchuk et al., 2018). Therefore, most patients underwent CMA analysis postnatally, either prior to or concurrently with RapSeq testing. The opinion of families related to sequencing approaches in the NICU is important. Although we did not systematically evaluate families' perception of RapSeq testing, they seemed to have an overall positive response. One family in particular who benefited from specific treatment seemed to display a markedly decreased level of anxiety which greatly facilitated interactions with clinical staff. One additional limitation was the small sample size. It also remains challenging to incontrovertibly establish cause-effect relationship between genomic results and predicted clinical phenotypes. For example, there is no certainty Patient 1 will develop Wilms tumor and concerns could be raised about the possibly subjecting an infant to an unnecessary ultrasound procedure. However, we felt it would be best for the patient to undergo screening. Another limitation is that only one case (Patient 2) was amenable to cost analysis comparison with a historic control, and our comparison was limited to normal variation of care in the NICU. A long-term limitation of ordering RapSeq in comparison to rGS is that if pathogenic variants are not identified to explain the phenotype, then, it is unlikely that the targeted gene panel will ever yield a diagnosis. Whereas with rGS, it is likely that some patients who do not receive a diagnosis at the time of initial testing may still receive one; this may occur if the undiagnosed patient's sequencing data is reanalyzed in the future when knowledge of gene-disease association has expanded.

Since implementing RapSeq, we have been able to offer parents the possibility of a timely unifying diagnosis for their acutely ill infants. We have shown multiple clinical benefits with potential total cost reduction when a rapid targeted gene panel is used. The success rate of RapSeq was at least as high as previously reported for rES and rGS (Soden et al., 2014; van Diemen et al., 2017). Moreover, the cost of RapSeq is about one half as much as rES and one third of rGS, and data interpretation is simplified by examining a panel of genes with known function. Thus, the implementation of RapSeq might facilitate the implementation of rapid sequencing tests in NICUs and PICUs, providing early prognostic data and more targeted therapeutic options to larger numbers of infants affected by Mendelian genetic disorders. This information can also provide pediatricians with additional prognostic data and screening recommendations to maximize the long-term potential of the infant. Despite encouraging results from various investigations, including ours, we caution against the unregulated use of rapid sequencing approaches outside carefully designed trials and/or pilot projects, possibly with a focus on infants admitted to level IV NICUs (Kapil, Fishler, Euteneuer, & Brunelli, 2019), until further analysis of clinical utility is provided through randomized trials.

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CONFLICT OF INTEREST

The authors have no financial relationships relevant to this article to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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