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Use of Exome Sequencing for Infants in Intensive Care Units: Ascertainment of Severe Single-Gene Disorders and Effect on Medical Management

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Abstract

Importance: While congenital malformations and genetic diseases are a leading cause of early infant death, the contribution of single-gene disorders in this group is undetermined.

Objective: To determine the diagnostic yield and utility of clinical exome sequencing in critically ill infants.

Design, setting, participants: Clinical exome sequencing was performed on 278 unrelated infants within the first 100 days of life, admitted to Texas Children's Hospital in Houston, over a period of five years, between December 2011 and January 2017. Exome sequencing types included proband exome, trio exome, and critical trio exome, a rapid genomic assay for seriously-ill infants.

Main outcomes and measures: Indications for testing, diagnostic yield of clinical exome sequencing, turnaround time, molecular findings, patient age at diagnosis, and impact on medical management in a group of critically ill infants suspected to have genetic disorders.

Results: Clinical indications for exome sequencing included a wide range of medical concerns. Overall, molecular diagnosis was achieved in 102/278 infants by clinical exome sequencing with a diagnostic yield of 36.7%. The diagnosis affected medical management in 53/102 (52.0%) of infants, with substantial impact on informed redirection of care, initiation of new subspecialist

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Author Contributions

Drs Lalani and Yang had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Study supervision: Lee, Eng, Yang, Lalani

Additional Contributions

We thank all families and referring physicians who submitted samples for testing.

Conflict of Interest Disclosures

The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from the clinical exome sequencing offered at the Baylor Genetics and the authors who are faculty members are indicated in the affiliation section.

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care, medication/dietary modifications, and furthering life-saving procedures in select patients. Critical trio exome revealed a molecular diagnosis in 32/63 infants (50.8%) at 33.1 ± 5.6 days of life with turnaround time (TAT) of 13.0 ± 0.4 days. Clinical care was altered by the diagnosis in 23/32 (71.9%) patients. The diagnostic yield, patient age at diagnosis, and medical impact in the group that underwent critical trio exome is significantly different comparing to regular exome testing. For deceased infants (n=81), genetic disorders were molecularly diagnosed in 39 (48.1%) by exome sequencing with implications for recurrence risk counseling.

Conclusions and relevance: Exome sequencing is a powerful tool for the diagnostic evaluation of critically ill infants with suspected monogenic disorders in the neonatal and pediatric ICUs, leading to notable impact on clinical decision-making.

Congenital malformations are estimated to be present in 13% of all admissions to neonatal intensive care units (NICUs) in the developed countries^{1,2} and remain the leading cause of neonatal mortality (estimated at 20–34%).^{3,4} While cytogenetic abnormalities⁵ and copy number variants (CNVs)⁶ are known causes of birth defects in seriously ill neonates, single-gene disorders are also significant contributors.^{7–11} The diagnostic tests for the clinical evaluation of newborns with suspected genetic diseases have expanded exponentially in recent years, particularly with the institution of the next-generation sequencing (NGS). As the overall burden of Mendelian disorders in neonates is being delineated via exome or whole genome sequencing (WGS) for newborn screening and other studies,^{12–14} clinical geneticists and neonatologists are in a unique position to initiate evidence-based studies in large tertiary care centers, deliver care that combines state of the art diagnostic tools and genetic counseling, and provide reproductive options regarding serious genetic diseases in at-risk families.

The clinical utility of rapid genome-wide sequencing was first demonstrated by Saunders *et al.* in 2012 in two neonates diagnosed by WGS within 50 hours¹⁵, and later by others in critically ill neonates or newborns, providing a diagnostic yield ranging from 40% to 57%.^{7,10} The need for a rapid comprehensive genetic diagnosis in ICUs for critically ill babies, especially those in level III and IV NICUs is paramount for both prognostication and clinical decision-making.^{8,16}

Here, we systematically evaluated the clinical utility of exome sequencing in the largest study sample to date in the ICU setting, of 278 unrelated infants < 100 days old from a single institution.

Methods

Clinical Samples

A total of 278 consecutive unrelated infants were retrospectively studied based on the following inclusion criteria: (1) age < 100 days of life at the time of testing; (2) referred from Texas Children's Hospital (Houston, TX) for exome sequencing from December 2011 to January 2017; and (3) exome sequencing performed at Baylor Genetics (Houston, TX) as a clinical service. Detailed clinical evaluation with comprehensive pre-test counseling was undertaken for all infants. The assessment for the need of clinical exome sequencing was carried out by multiple board-certified clinical geneticists at Texas Children's Hospital.

Relevant clinic notes were provided to the clinical laboratory. Parents provided consents for clinical exome testing with the option of receiving information on medically actionable findings and carrier status recommended by ACMG practice guidelines.^{17–19} The clinical aggregate data were collected with the approval of Baylor Institutional Review Board.

Exome Sequencing and Analysis

The 278 infants were studied by proband exome (available since December 2011), trio exome (available since October 2014), or critical trio exome (a rapid test available since April 2015) offered at Baylor Genetics as a clinical test and conducted as described.^{20,21} For this study, the average depth of coverage was 154X with 97.5% of the targeted regions (exonic regions of all nuclear genes plus $\pm$ 5bp of exon-intron boundaries) sequenced at 20X and higher (eTable 1 in the Supplement). All samples were concurrently analyzed by Illumina HumanOmni1-Quad or HumanExome-12 v1 array for quality-control, and detecting large copy-number variants (CNVs), absence of heterozygosity(AOH), and uniparental disomy (UPD). CNVs were also characterized using normalization of exome read depth as previously described.²² The procedures for regular and critical trio exome sequencing were highly similar except that critical exome cases were assigned a STAT test code and given the highest priority. Exome data were interpreted according to ACMG guidelines and variant interpretation guidelines of Baylor Genetics as previously described.^{20–23}

Molecular Diagnoses and Clinical Exome Reporting

Reporting of laboratory findings was performed as previously described.^{20,21} A case was classified as molecularly diagnosed when pathogenic or likely pathogenic variant(s) were detected in a disease gene associated with the phenotype observed in the studied individual; in addition, the zygosity of the mutant allele must match the inheritance pattern associated with the disease gene. For further validation, exome sequencing reports were additionally analyzed by board certified clinical geneticists in view of clinical correlation, follow-up evaluation, and confirmation of molecular diagnosis.

Human Phenotype Ontology (HPO) analysis

Clinical notes were rendered to HPO terms through BioLark natural language processing system and manual review.²⁴ Analyses were performed using Fisher tests to compare the diagnostic rate among patients annotated and under each top-branch HPO category. The False Discovery method (FDR) was used to transform Fisher p-values into q-values to address multiple testing across HPO terms.

Results

Demographics and Testing Indications

Of the 278 infants, 190 (68.3%) were in NICU at the time of sample submission, 43 (15.5%) were in the cardiovascular intensive care unit (CVICU), and 18 (6.5%) in the pediatric intensive care unit (PICU). There were 127 females (45%) and 151 males (55%), with a median age of 28 days at the time of sample submission (Table 1). Clinical indications for

exome sequencing included a wide range of clinical concerns (eTable 2 in the Supplement). Chromosomal microarray analysis (CMA) was completed in 237/278 (85%) infants.

Exome Sequencing Diagnoses in ICU

Exome sequencing method included proband exome (n=178), trio exome (n=37), or critical trio exome (n=63), depending on the availability of parental samples and tests at the time, and the overall cardiopulmonary status of the patients. There was no significant difference in patients' age and ICU admission rate at the time of testing among the three testing categories, although infants referred for critical exome were more likely to be in the ICU (61/63=96.8%) (Table 1).

Of the 278 infants, 102 individuals (36.7%) affected with 106 disorders, met criteria for molecular diagnosis (Table 1, Table 2 and eTable 3). Critical trio exome provided significantly higher molecular diagnoses in 32/63 infants (50.8%) than proband exome in 58/178 infants (32.6%) and trio exome in 12/37 cases (32.4%) ($p=0.011226$, Fisher's test). The median turnaround time was 13.0 days, shorter than that of proband exome (95.0 days) and trio exome (51.1 days) ($p<0.00001$, t-test). Consequently, the median age of diagnosis in infants getting critical exome (33.1 ± 5.6 days) was significantly younger than those undergoing proband or trio exome (116.5 ± 27.4 and 78.0 ± 103.1 days old, respectively) ($p=0.001883$, t-test).

Of the 102 solved cases, 56 (54.9%) had exome sequencing as a first-tier test (Table 3). For those individuals the average age at diagnosis was significantly younger than that in the others ($p=0.007907$, t-test). This is attributed to a younger age at test initiation, a greater proportion of patients undergoing critical trio exome, and a faster turnaround time with critical trio exome (Table 3).

Autosomal dominant, autosomal recessive and X-linked disorders were observed in 49 (46.2%), 44 (41.5%) and 13 (12.3%) infants respectively (Table 4). Four out of the 102 infants (3.9%) had dual molecular diagnoses (eTable 4 in the supplement). CNVs were detected in 11 individuals by NGS read depth and cSNP array; both are components of the exome assay (eFigure 1 in the Supplement). Of the diagnosed cases, *KMT2D*-related Kabuki syndrome (MIM: 147920), and Noonan spectrum disorders (MIM: 163950, 611553), caused by variants in *PTPN11* and *RAF1*, were observed in 8 (~8%) infants and comprise the most frequent single-gene diagnosis in the ICUs by exome. Both diseases presented in early infancy with significant cardiovascular abnormalities. Other genes found in at least 2 infants are summarized in eTable 5 in the Supplement, collectively comprising 11% (12/102) of the diagnoses in ICUs.

Approximately 39/102 (38%) of diagnosed individuals had atypical or unrecognized infantile presentation of genetic disorders. Of these, 4/39 (10%) infants were diagnosed with novel Mendelian diseases that were not reported initially and were only determined upon re-examination of the WES data; Some examples include that of an infant with severe hypertrophic cardiomyopathy and hypoglycemia caused by a *LZTR1* variant, and a neonate with congenital hypotonia and respiratory failure due to defect in *PURA*. For well known genetic disorders such as Kabuki syndrome caused by *KMT2D* mutation, craniofacial

features were atypical or under-recognized in all four infants. Some other examples of atypical presentation in neonates of known Mendelian disorders include *AKT3* related megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome in an individual with hypoglycemia, hyperlactatemia metabolic acidosis, and borderline prominent lateral ventricles without macrocephaly at birth, and *TUBA1A* mutation presenting as ventriculomegaly with fully formed corpus callosum.

To assess whether specific clinical presentations were more likely to be associated with molecular diagnosis, the diagnostic rate was compared among patients annotated with different phenotypes as represented by HPO terms. Analyses were performed at the top level branching of HPO phenotypes to ensure adequate counts of subjects. Individuals with phenotypes of the HPO category “abnormality of the cardiovascular system” (HP: 0001626) were found to be significantly under-represented in cases with molecular diagnosis ($p=0.000452$, FDR $q=0.0090$). “Abnormality of blood and blood-forming tissues” (HP: 0001871) and “abnormality of the musculature” (HP:0003011) were found to yield higher diagnostic rate ($p=0.00284$ and $p=0.00967$, FDR $q=0.028$ and $q=0.0644$, respectively) (Table 5).

Impact of Exome Sequencing on Clinical Management

We then evaluated the impact of molecular diagnosis by exome sequencing on medical management in four distinct areas: (a) redirection of care, (b) initiation of new subspecialist care, including additional diagnostic studies (c) changes in medication or diet, and (d) major procedures such as transplantation carried out in patients relevant to the genetic diagnoses. Using these considerations, we observed that molecular diagnoses directly affected medical management in 53/102 (52.0%) of patients after results were reported (Table 2 and eTable 3 in the Supplement). This rate is particularly higher in infants who received diagnosis through critical exome sequencing (23/32, 71.9%), compared to the other two groups that went through regular exome workup (30/70, 42.9%) (Table 1, $p=0.009902$). Of the positive cases in the critical trio exome group, a significantly higher portion (21/32 or 65.5%, $p<0.00001$) were diagnosed before discharge (Table 1).

Of the four aforementioned categories, first, informed redirection of care (including palliative care and withdrawal of life support) was undertaken in 19/53 (35.8%) infants (eTable 6 in the Supplement) with serious disorders such as muscular dystrophy-dystroglycanopathy type A, 7 [MIM:614643] (case 1247), and alveolar capillary dysplasia with misalignment of pulmonary veins [MIM:265380] (case 1028), and arterial calcification of infancy, generalized, 1 [MIM: 208000] (case 1202). Second, 27/53 infants (50.9%) benefitted from initiation of new subspecialist care, which was unanticipated prior to genetic testing. Examples include a diagnosis of aortic stenosis after cardiology evaluation in an infant with nephronophthisis and liver disease caused by compound heterozygous variants in *NPHP3* (case 1002). Similarly, diagnosis of short-rib thoracic dysplasia 3 with or without polydactyly [MIM:613091] in two infants allowed evaluation of renal, hepatic, pancreatic and ocular involvement in this ciliopathy-related disorder (case 1005, 1010). Third, dietary and medication changes likely impacted treatment of at least 7/53 infants (13.2%) including one with *ALDH7A1* related pyridoxine-dependent epilepsy (MIM:266100), who improved

significantly with cessation of seizures after high dose pyridoxine supplementation (case 1022). Another neonate with Menkes disease was started on copper histidine injections (case 1201). Lastly, major procedures such as transplantation were instituted in 5/53 infants (9.4%) who are all currently living. Hematopoietic stem cell transplantation (HSCT) was carried out in 3 infants; one with *RAG1* variants causing severe combined immunodeficiency (case 1021), another with *UNC13D* variants presenting with hemophagocytic lymphohistiocytosis (HLH) (case 1007), and a third infant with congenital pancytopenia due to defects in *FANCA* (case 1006). Cardiac transplantation was undertaken in an infant with a *PTPN11* variant presenting with severe concentric left ventricular hypertrophy after birth and severe pulmonic stenosis (case 1258), and another with left ventricular noncompaction (LVNC) due to a causal variant in *ACTC1* (case 1108).

Of the 102 infants who received a molecular diagnosis, 30 (29.4%) succumbed to their disease before 120 day of life (Table 1). In contrast, 28/170 (16.5%) infants in the undiagnosed group passed away ($p=0.014393$, Fisher test). Of all the deceased infants at the time of study ($n=81$), genetic disorders were confirmed in 39 (48.1%) by clinical exome.

Genetic Counseling

In addition to the medical impact to the patient self, exome sequencing also offer potential influence on the health management for family members and prevention of serious single-gene disorders in at-risk couples. Comprehensive genetic counseling was provided by a board-certified genetic counselor and/or clinical geneticists in 90/102 (88.2%) families who received a diagnosis. If an infant was deceased by the time the results were available, the parents were offered a follow-up counseling visit to discuss the genetic test results. Medically actionable secondary findings or carrier status were identified in 21 patients, among 267 families who agreed to receive this information (7.9%) (eTable 7 in the Supplement). Clinical exome sequencing diagnoses in infants directly affected parental health in at least two families: one with *BRCA2* variants revealing the genetic basis of cancer in both maternal and paternal sides of the family (case 1111); and another with *ACTC1* variant inherited from his father and paternal grandfather with diagnosis of pulmonary stenosis with ventricular septal defect and atrial septal defect, respectively (case 1108).

Partially Diagnosed and Negative Cases

Of the infants who were considered undiagnosed in this analysis, 4 infants received a partial diagnosis by exome sequencing, with relevant variants explaining only part of the phenotype (eTable 8 in the Supplement). Of the cases negative for exome results, one infant with neonatal hypotonia was diagnosed with myotonic dystrophy, detected by Southern blot analysis. Another was found to have infantile botulism.

Overall, 170/278 (61%) patients remained undiagnosed in this study. Clinical CMA a separate test, was done in 150/170 (~88%) of the undiagnosed infants, and no additional diagnoses were revealed by the analysis. In 85 out of 170 undiagnosed patients, mitochondrial genome sequencing was also performed which was non-diagnostic.

Discussion

We studied the impact of clinical exome sequencing in 278 infants predominantly in ICUs at a single tertiary institution in the first 100 days of life and ascertained 106 known disorders in 102 infants (with overall detection rate of 36.7%). Significantly higher detection rates with critical/rapid exome sequencing in seriously-ill infants have been shown in this study (50.8%, n=63), as well as in previous STATseq studies involving relatively smaller number of infants (57%, n=35).^{11,15} In our study, seriously ill infants were evaluated and selected for rapid exome study by clinical geneticists based on skilled clinical assessment. For the vast majority of infants selected for the rapid study, the indications included neuromuscular diseases, syndromic congenital cardiovascular malformations, hypertrophic cardiomyopathy with assessment for cardiac transplant; skeletal malformations and/or dysplasia; neonatal cholestasis and liver failure; lung disease including alveolar capillary dysplasia; cystic renal disease; and metabolic disorders with persistent lactic acidosis. This ascertainment likely allowed much greater probability of determining the underlying genetic cause for timely clinical management of very sick infants. Ultimately, the overall diagnostic rate of rapid exome sequencing would be driven by the eligibility screening of seriously-ill infants suspected to have genetic disorders, combined with institution-based cost concerns, and the practicability of obtaining rapid results for recognizable single gene-disorders.

Indications for clinical exome sequencing that were assessed to be of relatively low diagnostic yield by HPO phenotype analysis included isolated cardiovascular malformations, congenital diaphragmatic hernia in association with congenital heart defect (CHD), multiple congenital anomalies associated with maternal diabetes, and CHD with VACTERL association. On the other hand, HPO analysis determined a higher diagnostic rate for “abnormality of the musculature” phenotype, including hypotonia and joint contractures in this cohort. In another study, more complex phenotype has been noted to yield a higher diagnosis rate comparing to isolated phenotype.²⁵ Further studies in larger sample size are needed to corroborate these data for selecting infants most likely to benefit from exome sequencing in ICUs.

This study exposes a myriad of monogenic disorders that have been under-ascertained in critically ill neonates up until now.¹¹ While a comprehensive clinical evaluation is vital in allowing single-gene or panel testing in a subset of sick infants in the ICU, the power of NGS is indisputable in the expeditious detection of disorders that are clinically heterogeneous or complex due to dual diagnoses.²⁶ Every year, approximately 250 new monogenic disorders are described due to the escalating use of NGS.²⁷ The rapid pace of scientific advancement presents a considerable challenge, even to the most astute clinicians who provide care to infants suspected to have genetic disorders in critical care setting. While targeted testing is judicious in select cases, failure or delay in detecting causative variants in critically ill infants is a substantial concern, mitigated by exome sequencing. The atypical and unrecognized presentation of genetic disorders observed in about a significant 38% of these young infants further challenges the traditional paradigm of tiered genetic testing in critical care units.

Many qualities of exome sequencing that make it attractive as a clinical diagnostic tool, also present challenges for conducting traditional forms of economic evaluation of the service. In a recent study, performing exome sequencing as first-line test in infants achieved over three times diagnosis rate, with less than one-third of the cost, compared with simulated traditional tiered testing strategy of single gene or gene panels.²⁸ Additional studies on the cost-effectiveness are needed to inform both clinical and third party payers. For any individual patient, the cost-effectiveness of exome sequencing will differ according to the type of exome study performed, the point in the diagnostic pathway when exome sequencing is performed, and the particular genetic condition implicated. Analyses of data should aim to inform the clinical decision-making process through elucidation of the optimal role of sequencing for different groups of patients, taking both costs and impacts on clinical decision making as well as family planning into account. For example, the higher diagnostic yield from rapid exome testing should be considered alongside the higher associated cost for tests with reduced turnaround time. The cost to establish a diagnosis is of interest, as is the cost of exome sequencing as it relates to a health outcome. The most informative studies would provide evidence on the type of patient for whom exome sequencing is the most cost-effective form of diagnostic testing which leads to a molecular diagnosis and a change in care rendered according to the results.

Conclusion

Our study provides strong evidence that clinical exome sequencing uncovers monogenic disorders in a significant number of infants in NICU and PICU suspected to have genetic disorders, significantly impacting the medical care of over half of these diagnosed infants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Synnes AR, Berry M, Jones H, Pendray M, Stewart S, Lee SK. Infants with congenital anomalies admitted to neonatal intensive care units. *Am J Perinatol*. 2004;21(4):199–207. [PubMed: 15168318]
2. Widmann R, Caduff R, Giudici L, et al. Value of postmortem studies in deceased neonatal and pediatric intensive care unit patients. *Virchows Arch*. 2017;470(2):217–223. [PubMed: 27975126]
3. Hoyert DL, Freedman MA, Strobino DM, Guyer B. Annual summary of vital statistics: 2000. *Pediatrics*. 2001;108(6):1241–1255. [PubMed: 11731644]
4. Stevenson DA, Carey JC. Contribution of malformations and genetic disorders to mortality in a children's hospital. *Am J Med Genet A*. 2004;126A(4):393–397. [PubMed: 15098237]
5. Hudome SM, Kirby RS, Senner JW, Cunniff C. Contribution of genetic disorders to neonatal mortality in a regional intensive care setting. *Am J Perinatol*. 1994;11(2):100–103. [PubMed: 8198647]
6. Lu XY, Phung MT, Shaw CA, et al. Genomic imbalances in neonates with birth defects: high detection rates by using chromosomal microarray analysis. *Pediatrics*. 2008;122(6):1310–1318. [PubMed: 19047251]
7. Daoud H, Luco SM, Li R, et al. Next-generation sequencing for diagnosis of rare diseases in the neonatal intensive care unit. *CMAJ*. 2016;188(11):E254–260. [PubMed: 27241786]
8. Smith LD, Willig LK, Kingsmore SF. Whole-Exome Sequencing and Whole-Genome Sequencing in Critically Ill Neonates Suspected to Have Single-Gene Disorders. *Cold Spring Harb Perspect Med*. 2015;6(2):a023168. [PubMed: 26684335]

9. Soden SE, Saunders CJ, Willig LK, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Sci Transl Med*. 2014;6(265):265ra168.
10. Stark Z, Tan TY, Chong B, et al. A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. *Genet Med*. 2016;18(11):1090–1096. [PubMed: 26938784]
11. Willig LK, Petrikin JE, Smith LD, et al. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. *Lancet Respir Med*. 2015;3(5):377–387. [PubMed: 25937001]
12. Joseph G, Chen F, Harris-Wai J, Puck JM, Young C, Koenig BA. Parental Views on Expanded Newborn Screening Using Whole-Genome Sequencing. *Pediatrics*. 2016;137 Suppl 1:S36–46. [PubMed: 26729702]
13. Poulsen JB, Lescai F, Grove J, et al. High-Quality Exome Sequencing of Whole-Genome Amplified Neonatal Dried Blood Spot DNA. *PLoS One*. 2016;11(4):e0153253. [PubMed: 27089011]
14. Deem MJ. Whole-Genome Sequencing and Disability in the NICU: Exploring Practical and Ethical Challenges. *Pediatrics*. 2016;137 Suppl 1:S47–55. [PubMed: 26729703]
15. Saunders CJ, Miller NA, Soden SE, et al. Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. *Sci Transl Med*. 2012;4(154):154ra135.
16. Petrikin JE, Willig LK, Smith LD, Kingsmore SF. Rapid whole genome sequencing and precision neonatology. *Semin Perinatol*. 2015;39(8):623–631. [PubMed: 26521050]
17. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15(7):565–574. [PubMed: 23788249]
18. Grody WW, Thompson BH, Gregg AR, et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med*. 2013;15(6):482–483. [PubMed: 23619275]
19. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2017;19(2):249–255. [PubMed: 27854360]
20. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med*. 2013;369(16):1502–1511. [PubMed: 24088041]
21. Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA*. 2014;312(18):1870–1879. [PubMed: 25326635]
22. Lalani SR, Liu P, Rosenfeld JA, et al. Recurrent Muscle Weakness with Rhabdomyolysis, Metabolic Crises, and Cardiac Arrhythmia Due to Bi-allelic TANGO2 Mutations. *Am J Hum Genet*. 2016;98(2):347–357. [PubMed: 26805781]
23. Yang Y, Wang J, Xia F, et al. Adaptation of the ACMG/AMP Standards and Guidelines for Variant Interpretation: Experience within a Clinical Laboratory. 2016 American College of Medical Genetics Annual Meeting 2016.
24. Groza T, Kohler S, Doelken S, et al. Automatic concept recognition using the human phenotype ontology reference and test suite corpora. *Database (Oxford)*. 2015;2015.
25. Trujillano D, Bertoli-Avella AM, Kumar Kandaswamy K, et al. Clinical exome sequencing: results from 2819 samples reflecting 1000 families. *Eur J Hum Genet*. 2017;25(2):176–182. [PubMed: 27848944]
26. Posey JE, Harel T, Liu P, et al. Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation. *N Engl J Med*. 2017;376(1):21–31. [PubMed: 27959697]
27. Chong JX, Buckingham KJ, Jhangiani SN, et al. The Genetic Basis of Mendelian Phenotypes: Discoveries, Challenges, and Opportunities. *Am J Hum Genet*. 2015;97(2):199–215. [PubMed: 26166479]
28. Stark Z, Schofield D, Alam K, et al. Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. *Genet Med*. 2017.

Key Points

Question:

What is the clinical utility of exome sequencing, when employed in neonatal and pediatric intensive care units?

Findings:

In this retrospective study of 278 infants within the 100 days of life referred for clinical exome sequencing, 36.7% received a genetic diagnosis, with medical management impacted in 52.0% of the diagnosed patients; critical trio exome testing yielded a higher diagnostic rate (50.8%) at an earlier age (TAT 13.0 ± 0.4 days), and are more likely to impact medical management (71.9%).

Meaning:

Utilizing exome sequencing in intensive care units can significantly impact the medical care of critically ill infants suspected to have genetic disorders.

Table 1

Summary of 278 infants tested with exome sequencing

Patient Information		Overall Rate (n=278)	Proband exome (n=176, 63%)	Trio exome (n=39, 14%)	Critical trio exome (n=63, 22%)	p value*** (critical trio exome vs the rest)
Demographic	Patient age (days) Median $\pm$ sem *	28.5 $\pm$ 1.7	29.0 $\pm$ 2.2	31.5 $\pm$ 3.9	22.7 $\pm$ 3.9	0.521667
	Number and percentage of patients in ICU	251/278 (90.3%)	156/176 (88.6%)	34/39 (89.5%)	61/63 (96.8%)	0.052420
Exome sequencing	Exome sequencing diagnosis (number and percentage)	102/278 (36.7%)	57/176 (32.4%)	13/39 (33.3%)	32/63 (50.8%)	0.011226
	TAT (days) Median $\pm$ sem	73.1 $\pm$ 2.1	95.0 $\pm$ 1.5	51.1 $\pm$ 3.2	13.0 $\pm$ 0.4	<0.00001
Medical impact	ICU stay length (days) Median $\pm$ sem	29.5 $\pm$ 5.1	28.0 $\pm$ 6.3	32.0 $\pm$ 14.3	42.5 $\pm$ 10.2	0.112285
	5-year death rate (number and percentage)	38.5 $\pm$ 4.6	41.0 $\pm$ 5.8	35.0 $\pm$ 6.9	31.0 $\pm$ 13.4	0.834061
	120-day death rate (number and percentage)	39/102 (38.2%)	27/57 (47.4%)	2/13 (15.4%)	10/32 (31.3%)	0.384029
	In diagnosed patients with exome sequencing	41/170 (24.1%)	30/117 (25.6%)	3/25 (12.0%)	8/28 (28.6%)	0.629165
	Age at diagnosis Median $\pm$ sem	30/102 (29.4%)	18/57 (31.6%)	2/13 (15.4%)	10/32 (31.3%)	0.817358
	Diagnosed before discharge (number and percentage)	28/170 (16.5%)	21/117 (17.9%)	1/25 (4.0%)	6/28 (21.4%)	1
	Affected medical management? (number and percentage)-Total	94.4 $\pm$ 21.0	116.5 $\pm$ 27.4	78.0 $\pm$ 103.1	33.1 $\pm$ 5.6	0.001883
	Re-direction of care	38/102 (37.3%)	13/57 (22.8%)	4/13 (30.8%)	21/32 (65.6%)	<0.00001
	Initiation of subspecialist care	53/102 (52.0%)	26/57 (45.6%)	4/13 (33.3%)	23/32 (71.9%)	0.009902
	Change in treatment or diet	19	11	0	8	NA
	Major procedures completed	27	12	3	12	NA
	Change in treatment or diet	7	2	1	4	NA
	Major procedures completed	5	2	0	3	NA

* sem: standard error of mean (sem)

** Excluding partial diagnosis or diagnosed by other methodologies

*** two-tailed t-test or Fisher exact test, when applicable. Bold, p value < 0.05

Table 2

Clinical impact of critical trio exome in 32 patients received diagnosis

ID	Gender	Age at testing (days)	Exome sequencing TAT (days)	Gene(s)	Disease(s)	Inheritance pattern	Variants	Zygosity	Status	Impact on Clinical Management	Exome sequencing as first-tier test	Exome sequencing returned before discharge/death
1002	M	57	11	<i>NPHP3</i>	Nephronophthisis 3 [MIM:604387]; Renal-hepatic-pancreatic dysplasia 1 [MIM:208540]	AR	c.1928C>T (p.P643L), c.2694_2694-1delAG	compound het	Alive	Cardiology follow up for mild aortic valve stenosis and mildly hypoplastic pulmonary valve annulus	Y	Y
1004	M	18	14	<i>HSD17B4</i>	D-bifunctional protein deficiency [MIM:261515]; Perrault syndrome 1 [MIM:233400]	AR	c.1210-11C>G, c.936_937delTA (p.T313*)	compound het	Alive	Endocrinology evaluation for adrenal insufficiency; audiology and gastroenterology referral	Y	N
1005	M	5	9	<i>DYNC2H1</i>	Short-rib thoracic dysplasia 3 with or without polydactyly [MIM: 613091]	AR	c.10594C>T (p.R3532*), c.9814T>A (p.L3272I)	compound het	Alive	Follow up for renal, hepatic, pancreatic and ocular disease for future concerns	Y	Y
1006	M	9	10	<i>FANCA</i>	Fanconi anemia, complementation group A [MIM:227650]	AR	c.154C>T (p.R52*), c.2852G>A (p.R951Q)	compound het	Alive	Bone marrow transplant for Fanconi anemia	Yes, concurrent with breakage studies	Y
1007	M	81	15	<i>UNC13D</i>	Hemophagocytic lymphohistiocytosis, familial, 3 [MIM:608898]	AR	c.118-308C>T (N/A), c.2346_2349delGGAG (p.R782fs)	compound het	Alive	Bone marrow transplant for hemophagocytic lymphohistiocytosis	Yes, concurrent with CMA	Y
1008	M	10	12	<i>ACAD9</i>	Mitochondrial complex I deficiency due to ACAD9 deficiency [MIM: 611126]	AR	c.163C>T (p.P55S), c.860G>A (p.G287E)	compound het	Alive	Follow up with Cardiology; Riboflavin	Y	N
1009	M	89	14	<i>LIPT1</i>	Lipoyltransferase 1 deficiency [MIM:616299]	AR	c.212C>T (p.S71F), c.539T>C (p.L180S)	compound het	Alive	Redirection of care	Y	Y
1011	M	26	9	<i>KLHL40</i>	Nemaline myopathy 8, autosomal recessive [MIM:615348]	AR	c.472_475delCGCT (p.A158fs), c.1153-2A>T	compound het	Alive	N/A	Y	Y
1012	M	91	14	<i>SLC4A11</i>	Corneal dystrophy, Fuchs endothelial, 4 [MIM:613268]	AR	c.1040G>A (p.R347Q), c.1855G>A (p.A619T)	compound het	Alive	N/A	N	Y
1013	F	34	13	<i>COL12A1</i>	Ullrich congenital muscular dystrophy-2 [MIM: 616470]; Bethlem myopathy 2 [MIM: 616471]	AR	c.5794+2T>A (N/A), c.5269C>T (p.R1757*)	compound het	Alive	N/A	Yes, concurrent PWS, SMA and Trio WES	Y
1014	M	34	13	<i>CASK</i>	FG syndrome 4 [MIM:300422]; Mental retardation and microcephaly with pontine and	XL	c.1721dupA (p.S575fs)	<i>de novo</i> hemi	Alive	Redirection of care	Y	Y

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ID	Gender	Age at testing (days)	Exome sequencing TAT (days)	Gene(s)	Disease(s)	Inheritance pattern	Variants	Zygosity	Status	Impact on Clinical Management	Exome sequencing as first-tier test	Exome sequencing returned before discharge/death
					cerebellar hypoplasia [MIM: 300749]							
1015	M	7	12	<i>EFTUD2</i>	Mandibulo-facial dysostosis, Guion-Almeida type [MIM:610536]	AD	c.869+1G>C	<i>de novo</i> het	Alive	Audiology in addition to multiple subspecialties already involved in care	Y	Y
1016	M	97	14	<i>SMARCA4</i>	Coffin-Siris syndrome 4 [MIM: 614609]	AD	c.2936G>A (p.R979Q)	<i>de novo</i> het	Alive	N/A	N	Y
1024	M	7	14	<i>CHD7</i>	CHARGE syndrome [MIM:214800]	AD	c.7234G>T (p.E2412X)	<i>de novo</i> het	Alive	Ophthalmology and Immunology evaluation	Y	Y
1026	M	18	19	<i>KCNQ2</i>	Epileptic encephalopathy, early infantile, 7 [MIM:613720]; Seizures, benign familial neonatal 1 (BFNS1)	AD	c.1742G>A (p.R581Q)	<i>de novo</i> het	Alive	Developmental therapies initiated sooner due to association with EIEE7 and BENE	Y	N
1030	M	11	13	<i>KMT2D</i>	Kabuki syndrome 1 [MIM:147920]	AD	c.13040_13041del (p.Q43+7fs)	<i>de novo</i> het	Alive	Immunology and Ophthalmology evaluation	N	N
1052	M	4	17	Xp22.31p22.33 loss		XL	chrX:181779-8997440 loss	Inherited hemi	Alive	initiating endocrine work up-found to have hypogonadotropic hypogonadism; mother previously had multiple miscarriages-found to carry the deletion	Yes, concurrent with CMA	Y
1104	F	7	10	<i>KLHL24</i>	Epidermolysis bullosa (EB)	AD	c.1A>G (p.M1?)	<i>de novo</i> het	Alive	Facilitated appropriate management by Dermatology for newly described (2016) epidermolysis bullosa simplex form	Y	N
1105	M	36	15	<i>WNT5A</i>	Robinow syndrome, autosomal dominant 1 [MIM:180700]	AR	c.496C>T (p.R166C)	homo	Alive	N/A	N	N
1106	F	10	14	<i>MUT</i>	Methylmalonic aciduria, mut(0) type [MIM:251000]	AR	c.422C>A (p.A141E)	homo	Alive	Pro-Phree/Propimex-1 formula and carnitine	Yes, concurrent with metabolic panels	Y
1108	M	58	17	<i>ACTC1</i>	Left ventricular noncompaction 4; Cardiomyopathy, dilated, 1R [MIM: 613424]; Cardiomyopathy,	AD	c.635G>A (p.R212H)	Inherited het (from father)	Alive	Orthotopic heart transplant for left ventricular non-	Yes, concurrent with CMA	Y

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ID	Gender	Age at testing (days)	Exome sequencing TAT (days)	Gene(s)	Disease(s)	Inheritance pattern	Variants	Zygosity	Status	Impact on Clinical Management	Exome sequencing as first-tier test	Exome sequencing returned before discharge/death
					hypertrophic, 11 [MIM:612098]; Atrial septal defect 5 [MIM:612794]					compaction cardiomyopathy		
1111	F	7	19	<i>BRCA2</i>	Fanconi anemia, complementation group D1 [MIM:605724]; Breast-ovarian cancer, familial, 2 [MIM: 612555]	AR	c.4965C>G (p.Y1655*), c.7007G>C (p.R2336P)	compound het	Deceased	N/A (see notes)	Yes, concurrent with CMA	Y
1116	F	15	13	<i>TRMU</i>	Liver failure, transient infantile [MIM:613070]	AR	c.117G>A (p.W39*), c.680G>C (p.R227T)	compound het	Deceased	Redirection of care	N	N
1173	F	83	13	<i>FAT4</i>	Van Maldergen syndrome 2 (VMLDS2) [MIM:615546]	AR	c.739C>A (p.P247T), c.2486T>G (p.L829R)	compound het	Alive	Audiology evaluation, in addition to multiple subspecialties already involved in care	Y	Y
1198	F	14	13	<i>ETFDH</i>	Glutaric acidemia IIC [MIM: 231680]	AR	c.405+3A>G (N/A), c.739G>C (p.G247R)	compound het	Deceased	Treated with Carbaglu (carglumic acid) and Riboflavin; Redirection of care	N	Y
1202	F	14	10	<i>ENPP1</i>	arterial calcification of infancy, generalized, 1 (GACI1) [MIM: 208000]	AR	c.913C>A (p.P305T); c.2246C>G (p.S749*)	compound het	Deceased	Treated with panidronate; Redirection of care	Y	Y
1204	M	50	13	<i>PTPN11</i>	LEOPARD syndrome 1 [MIM: 151100]; Metachondromatosis [MIM:156250]; Noonan syndrome 1 [MIM:163950]	AD	c.1528C>G (p.Q510E)	<i>de novo</i> het	Deceased	N/A	Yes, concurrent with CMA	Y
1207	M	6	15	<i>DYNC1H1</i>	Mental retardation, autosomal dominant 13 [MIM:614563]	AD	c.6074G>A (p.R2025Q)	<i>de novo</i> het	Deceased	N/A	Yes, concurrent with CMA	N
				<i>KMT2C</i>	Kleefstra syndrome [MIM: 610253]	AD	c.4513A>G (p.I1505V)	<i>de novo</i> het				
1209	M	86	24	<i>OFD1</i>	Joubert syndrome 10 [MIM: 300804]; Orofaciodigital syndrome I [MIM:311200]	XL	c.604_609del (p.E202_Y203del)	Inherited hemi	Deceased	Redirection of care	N	N
1210	M	32	9	<i>GBE1</i>	Glycogen storage disease IV [MIM: 232500]	AR	c.1239delT (p.D413fs)	homo	Deceased	Redirection of care	Yes, concurrent with CMA	N
1217	M	7	13	8p23.3p23.1 loss; 12p13.33p13.31 gain	Unbalanced translocation	AD	chr8:190907 – 8234192 loss; chr12:234929 – 8376765 gain	<i>de novo</i> het	Deceased	N/A	Yes, concurrent with CMA	Y
1226	M	43	13	11q23.3q25 gain;22q11.1q11.21 gain	Emmanuel syndrome	AD	chr11:116691675 – 13489485 gain; chr22:17072086–20130474 gain	<i>de novo</i> het	Deceased	Redirection of care	N	N

Notes: While there was no direct clinical impact on the infant, cascade testing directly affecting parental health was relevant
Het: heterozygous; Hemi: hemizygous; homo: homozygous

Table 3
Summary of cases receiving molecular diagnosis with exome as first-tier or second-tier testing

	Patient age at testing (days) median±sem	Exome TAT (days) median±sem	Patient age at diagnosis (days) median±sem	Clinical management changed (number and percentage)	Exome category (number and percentage)
Exome offered as first-tier testing 56/102 (54.9%)	13.7±3.9	38.4±4.7	70.8±20.6	30/56 (53.6%)	Proband: 25/56 (44.6%) Trio: 7/56 (12.5%) Critical: 24/56 (42.9%)
Exome offered as second-tier testing 46/102 (45.1%)	36.6±4.4	73.0±5.1	123.6±37.6	23/46 (50.0%)	Proband: 33/46 (71.7%) Trio: 5/46 (10.8%) Critical: 8/46 (17.4%)
<i>p</i> value (two-tailed t-test)	0.004904	0.001013	0.007907	0.842402	N/A

sem: standard error of mean

N/A: not applicable

Table 4

Summary of the molecular diagnoses provided by exome sequencing

Category		Number of diagnoses	%
* Autosomal dominant	<i>De novo</i>	36 (4)	34.0
	Inherited	5	4.7
	Inheritance unknown	8 (4)	7.5
* Autosomal recessive	Compound heterozygous	29	27.4
	Homozygous	6	5.7
	Phase unknown	9	8.5
* X-linked hemizygous	<i>De novo</i>	6 (2)	5.6
	Carrier mother	6 (1)	5.6
	Carrier mother (mosaic)	1	0.9
Total		106 (from 102 individuals)	

* Causal variants are point variants, small indels, or large CNVs. Number in parenthesis indicates cases with large CNV findings.

Table 5
Comparison of diagnostic rate by exome sequencing in groups with and without the phenotype

HPO term	HPO ID	Diagnostic rate in individuals with the term	Diagnostic rate in individuals without the term	Odds ratio [95% CI]	p-value	FDR q-value
abnormality of the cardiovascular system	HP:0001626	39/141 (27.7%)	63/130 (48.5%)	0.408 [0.238,0.694]	0.000452	0.009047
abnormality of blood and blood-forming tissues	HP:0001871	17/26 (65.4%)	85/245 (34.7%)	3.537 [1.42,9.415]	0.002838	0.02838
abnormality of the musculature	HP:0003011	31/59 (52.5%)	71/212 (33.5%)	2.191 [1.173,4.119]	0.009673	0.064487
growth abnormality	HP:0001507	24/49 (49.0%)	78/222 (35.1%)	1.768 [0.902,3.467]	0.075351	0.322381
abnormality of metabolism/homeostasis	HP:0001939	31/66 (47.0%)	71/205 (34.6%)	1.668 [0.913,3.044]	0.080595	0.322381
abnormality of the skeletal system	HP:0000924	43/99 (43.4%)	59/172 (34.3%)	1.468 [0.857,2.516]	0.152758	0.445911
abnormality of connective tissue	HP:0003549	7/28 (25.0%)	95/243 (39.1%)	0.520 [0.18,1.333]	0.156481	0.445911
abnormality of the endocrine system	HP:0000818	3/15 (20.0%)	99/256 (38.7%)	0.397 [0.07,1.524]	0.178365	0.445911
abnormality of head or neck	HP:0000152	52/125 (41.6%)	50/146 (34.2%)	1.366 [0.81,2.308]	0.257673	0.572606
abnormality of the nervous system	HP:0000707	42/100 (42.0%)	60/171 (35.1%)	1.338 [0.781,2.29]	0.298829	0.597659
abnormality of the integument	HP:0001574	12/26 (46.2%)	90/245 (36.7%)	1.473 [0.594,3.603]	0.396455	0.663676
abnormality of the immune system	HP:0002715	7/14 (50.0%)	95/257 (37.0%)	1.701 [0.493,5.878]	0.398206	0.663676
abnormality of the eye	HP:0000478	5/17 (29.4%)	97/254 (38.2%)	0.675 [0.181,2.138]	0.607963	0.935328
abnormality of the abdomen	HP:0001438	29/73 (39.7%)	73/198 (36.9%)	1.128 [0.624,2.023]	0.673953	0.96279
abnormality of prenatal development or birth	HP:0001197	7/17 (41.2%)	95/254 (37.4%)	1.170 [0.365,3.54]	0.79883	0.985792
abnormality of the ear	HP:0000598	10/25 (40.0%)	92/246 (37.4%)	1.115 [0.429,2.783]	0.830338	0.985792
abnormality of the genitourinary system	HP:0000119	26/71 (36.6%)	76/200 (38.0%)	0.942 [0.514,1.707]	0.887213	0.985792
abnormality of the respiratory system	HP:0002086	23/62 (37.1%)	79/209 (37.8%)	0.970 [0.513,1.807]	1	1
abnormality of limbs	HP:0040064	17/46 (37.0%)	85/225 (37.8%)	0.965 [0.468,1.943]	1	1