Genetics inMedicine | BRIEF REPORT

Exome sequencing in neonates: diagnostic rates, characteristics, and time to diagnosis

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Purpose: Neonatal patients are particularly appropriate for utilization of diagnostic exome sequencing (DES), as many Mendelian diseases are known to present in this period of life but often with complex, heterogeneous features. We attempted to determine the diagnostic rates and features of neonatal patients undergoing DES.

Methods: The clinical histories and results of 66 neonatal patients undergoing DES were retrospectively reviewed.

Results: Clinical DES identified potentially relevant findings in 25 patients (37.9%). The majority of patients had structural anomalies such as birth defects, dysmorphic features, cardiac, craniofacial, and skeletal defects. The average time for clinical rapid testing was 8 days.

Conclusion: Our observations demonstrate the utility of family-based exome sequencing in neonatal patients, including familial cosegregation analysis and comprehensive medical review.

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Key Words: exome; genetic testing; neonatal; neonatal intensive care unit; NICU

INTRODUCTION

Since 2011, diagnostic exome sequencing (DES) has proven cost-effective and beneficial in providing molecular diagnoses for patients with a broad spectrum of previously undiagnosed genetic diseases and broadening the phenotype of known genetic diseases.[1] The application of DES enables many undiagnosed patients who have endured extensive genetic testing to receive a definitive genetic diagnosis and enables other patients to receive a diagnosis earlier than with traditional genetic-testing methods.

Neonatal patients are particularly appropriate for utilization of DES as many Mendelian diseases are known to present in this period but often with nonspecific, heterogeneous features, rendering less useful traditional testing which requires a clinical diagnosis or differential diagnosis.[2] Over 20% of infant deaths in the United States are caused by chromosomal abnormalities, congenital malformations, and deformations.[3] Despite this, the exact incidence of Mendelian disease in neonates is uncertain. Traditional sequential gene sequencing is not expedient in neonates owing to high cost, turnaround times, and heterogeneity of phenotypes at this young age. Nevertheless, diagnosis in neonatal patients leads to more effective treatments, identification at preventative screenings, and management.[4–6] While case studies have shown that DES can provide diagnoses in a small number of neonatal and fetal patients with structural anomalies, only a few studies have focused on neonatal patients.[7,8] While diagnosis of a genetic condition can aid in treatment, prognosis, and decision-making in neonatal patients, until the availability of DES, reaching a diagnosis was difficult owing to significant heterogeneity in presentation within this time period. Herein we investigate DES in a diverse neonatal population (from birth to 1 month of age at the time of testing), demonstrate a rate of 37.9% potentially relevant findings, and include a case study to illustrate how DES may aid in diagnosis and treatment of an affected neonate within the first month of life.

MATERIALS AND METHODS

Subjects

Fifty-six neonatal patients (from birth to 1 month old at the time of testing) were identified sequentially through clinical samples sent to Ambry Genetics Laboratory (Aliso Viejo, CA) for DES. The Solutions Institutional Review Board determined the study to be exempt from the Office for Human Research Protections Regulations for the Protection of Human Subjects (45 Code of Federal Regulations 46) under category 4. Patients were consented for testing by the ordering provider. An additional 10 neonatal patients were referred for research testing through a university medical center. Parental

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and/or additional informative family member samples (when available) were also sent for these patients and utilized for variant phasing and analysis and cosegregation analysis.

Testing methods

Diagnostic exome sequencing

All patients' clinical and testing histories, along with pedigrees provided by referring physicians, were reviewed and summarized by a team of board-certified genetic counselors with previous clinical experience. DNA isolation, exome library preparation, sequencing, bioinformatics, and data analysis were performed as previously described.[9,10] Genes were classified as characterized (known to cause Mendelian disease) or uncharacterized (not previously associated with disease) based on Ambry's clinical validity assessment criteria.[11]

For rapid cases (exome ordered with accelerated turn around time), careful review was performed using the Integrative Genomics Viewer so that verbal results could be given before Sanger confirmation. All relevant alterations were confirmed by Sanger sequencing.

Secondary findings

Secondary or incidental findings (SF) are described by ACMG as the "results of a deliberate search for pathogenic or likely pathogenic alterations in genes that are not apparently relevant to a diagnostic indication for which the sequencing test was ordered." Secondary findings for the ACMG list of genes was an option for patients at no charge once ACMG established guidelines.

Statistical analysis

Secondary findings results were compared between nonneonate and neonatal patients using the Fisher's exact test.

RESULTS

Characteristics of neonatal patients

A total of 66 patients 1 month of age and under were identified for evaluation. Demographic characteristics of these patients are summarized in **Table 1**. Twenty-seven patients did not have uncharacterized genes analyzed, owing to the lack of an informative trio for DES, clinician order (opted out of novel gene analysis), or a positive finding in a characterized gene. The average time from initiation of testing to report was 72 days, for cases within the past 2 years 61 days. The average time, in those who elected clinical rapid testing, until the health-care provider was notified of a result was 8 days, and for Sanger confirmation and a written report it was 15 days.

Thirty-three patients (50%) had no previous postnatal genetic testing reported, 26 (39.4%) had a postnatal chromosome microarray, 12 (18.2%) had a postnatal karyotype and 4 (6.1%) had a postnatal single-gene test or gene panel. None of these tests were reported as diagnostic.

Patients with relevant findings

Clinical DES identified potentially relevant findings in 32 of the patients (32/66, 48.5%), with 25/66 (37.9%) having positive or likely positive findings (**Table 2**). Fourteen patients had positive findings in characterized genes (*ACTG2, ASXL1, CLPB, FBXL4, GNB5, HNF4A, LRP5, MAGEL2, NOTCH1, NSD1, RAB23, RECQL4, SF3B4*, and *TUBB3)*. Eleven patients had likely positive findings in characterized genes *(ACTA1, COL2A1, EP300, MYBPC3, PNKP, RBM10, RYR1, SMARCA4, SON, SOX10*, and *XYLT1)*. Six patients had uncertain findings (variant of

Table 1 Demographic information

Characteristic	Number of probands (n = 66)
Gender	
Male	41 (62.1%)
Female	25(37.9%)
Ethnicity	
Caucasian	22 (33.3%)
Hispanic	15 (22.7%)
Multiple ethnicities	13 (19.7%)
African American	5 (7.6%)
Asian	4 (6.1%)
Middle Eastern	2 (3.0%)
Unknown/other	2 (3.0%)
Ashkenazi Jewish	2 (3.0%)
Jamaican	1 (1.5%)
Clinical history[a]	
Multiple congenital anomalies	38 (57.6%)
Dysmorphic features	21 (31.8%)
Abnormal brain MRI	19 (28.8%)
Failure to thrive/undergrowth	12 (18.1%)
Hypotonia	9 (13.6%)
Seizures/epilepsy	8 (12.1%)
Progressive phenotype	7 (10.6%)
Intellectual disability/developmental delay	5 (7.6%)
Overgrowth	3 (4.5%)
Organ system involvement[a]	
Cardiovascular	33 (50.0%)
Neurologic	30 (45.5%)
Musculoskeletal/structural	29 (43.9%)
Craniofacial	25 (37.9%)
Gastrointestinal	19 (28.8%)
Pulmonary	18 (27.3%)
Metabolic/biochemical	13 (19.7%)
Renal	16 (24.2%)
Ophthalmologic	12 (18.1%)
Genitourinary	11 (16.7%)
Endocrine	7 (10.6%)
Hematologic	5 (7.6%)
Dermatologic	4 (6.1%)
Audiologic/otolaryngologic	3 (4.3%)
Allergy/immunologic/infectious	2 (3.0%)
Dental	2 (3.0%)
Oncologic	1 (1.5%)

[a]Figures do not add up to 100% because some patients have multiple findings.

Table 2 Diagnostic results

Category	Diagnostic rates
All cases	25/66 (37.9%)
Trios	22/58 (37.9%)
Clinical rapid cases	3/6 (50.0%)
All clinical cases	24/56 (42.9%)

uncertain significance or uncertain gene phenotype overlap) in characterized genes (*DPH1, DYNC2H1, EFTUD2, MYH8, PKHD1*, and *RYR1*). One patient had novel candidate gene findings *(HHAT*) (**Supplementary Table S1 online**).

Of the positive/likely positive findings, 15/25 (60%) were in autosomal dominant genes, 10/14 (71.4%) were de novo, 4/14 (28.6%) were inherited, and the remainder unknown. Also of the positive/likely positive cases, 7/25 (28.0%) were in autosomal recessive genes, with 6/7 (85.7%) inherited, the remainder unknown; and only one case had reported consanguinity. Also of those cases, one was X-linked recessive (inherited), one X-linked dominant (de novo), and another case had complex inheritance (inherited).

One case was reclassified based on new literature as having findings in two genes post initial reporting (alteration in *GNB5* added).

Secondary findings

Of the 66 patients, 17 (25.7%) did not have/declined SF testing, which was a significantly higher figure than that of nonneonatal probands during the same time period ($p = 0.0001$). Forty-eight patients had negative SF results, and one of the patients requesting ACMG SF testing had positive results in *LDLR* (2.1%).

Neonatal case report

The male proband was born at 38 5/7 weeks (birthweight 2.66 kg, length 48 cm, occipital frontal circumference 35.7 cm) after a pregnancy complicated by intrauterine growth restriction and decreased fetal movements. A cesarean section was recommended because of the maternal condition. Apgar scores were 2, 3, and 4 at 1, 5, and 10 minutes, respectively, owing to low respiratory response and very limited spontaneous movement. He was also noted to have a fractured humerus. He was found to have doughy muscles with decreased muscle bulk and had a high arched palate connected to a small midline defect in the alveolar ridge where the frenulum attaches. He had a persistently elevated hemidiaphragm, but imaging studies were normal.

The maternal and family histories were complex. The mother was diagnosed with Moebius syndrome with ophthalmoplegia at age 5, and in early adulthood was found to have an unknown but progressive polyneuropathy and multiple sclerosis. She was reported to be legally blind with hearing loss and a white forelock. The mother's *TUBB3* gene sequencing was normal. A maternal half-brother of the proband died shortly after birth at 35 weeks gestation and was said to have fetal akinesia deformation sequence. There was severe intrauterine growth restriction and very poor fetal movement. An autopsy confirmed features consistent with Pena Shokeir/fetal akinesia deformation sequence and absence of the olfactory nerves. No consanguinity was reported.

Results of a karyotype, SNP array, *DM1* testing, and testing for spinal muscular atrophy were normal. Other imaging studies were nondiagnostic. Rapid DES was ordered to aid in prognosis and counseling, and revealed a likely pathogenic *SOX10* c alteration, c.523C > T (p.P175S). *SOX10* alterations are associated with peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome with or without Hirschsprung disease (OMIM 609136). This alteration was absent from the unaffected father and present in the mother. The alteration was not present in four databases (the National Heart, Lung, and Blood Institute Exome Sequencing Project, 1000 Genomes, ExAC, or the Database of Single Nucleotide Polymorphisms) and is predicted to be probably damaging by Polyphen and deleterious by SIFT.[12–17] The alteration is located in a functionally important protein domain and in a mutation hot spot where similar amino-acid changes have been observed.[18]

Identification of the *SOX10* alteration led to challenging discussions with the family regarding life on permanent ventilator support or the option of withdrawing support and care. Of the few reported cases, many of the infants have been noted to have central hypoventilation or other respiratory depression without any recovery of function. While it was suggested in Touraine et al. that intrafamilial variability might exist, this is the first case of intrafamilial variability reported.[19] After much debate, thought, and discussion, the hard decision to withdraw ventilator support, given the poor prognosis and expected outcome, was made. Based on the information obtained in this pregnancy via whole-exome sequencing, prenatal testing was conducted in a subsequent pregnancy where the fetus was found not to carry the alteration. This pregnancy resulted in a 37-week healthy male fetus delivered by planned cesarean section (given maternal history).

DISCUSSION

Genetic testing in neonates is not unusual, and benefits in medication use, procedures, diet, treatment and surveillance plans, prognosis, and counseling have commonly been reported in both patients diagnosed in the neonatal intensive care unit and subsequent diagnoses.[4,20] Initial concerns over the time taken to return results have been eliminated with the use of rapid exome sequencing. DES is now faster than single-gene testing. The ability of a health-care provider to order testing and counsel families within 8 days is advantageous for neonatal patients, especially as 37.9% received a diagnosis. If the patient develops additional symptoms or new literature regarding previously unknown syndromes is published, exome reanalysis and reclassification may later provide an accurate diagnosis.

Interestingly, the diagnostic rates amongst nontrios and trios are the same. Although there are few nontrios (perhaps owing to increased availability of parents in the neonatal

period), it is plausible to suggest that overall neonates are overall more likely to receive a diagnosis. While most studies have shown trio testing to be advantageous for DES, perhaps this effect is limited to those outside the neonatal period.[1]

Over half of patients did not undergo genetic testing prior to DES. This suggests that it is increasingly becoming a first-line test, especially in neonates. The majority of those undergoing genetic testing did not have these tests performed concurrently, which conceivably might reduce the time to diagnosis. The single-gene testing and panel tests performed in this cohort have a laboratory-quoted turnaround time longer than that of DES. Therefore, we suggest that in comparable genes/testing modalities in neonates, DES should be considered rather than single-gene testing, to decrease time to diagnosis.

Interestingly, more families of probands declined SF during this time period. This may be due to the critical health of the patient during this time period. Perhaps parents felt that they could not cope emotionally with additional information beyond the current health problems, or perhaps it was due to the young age of the patients. Further studies could be undertaken to determine the reasons for this finding.

The majority of features seen in neonatal patients included multiple structural anomalies such as birth defects, dysmorphic features, cardiac, craniofacial, and skeletal defects. This is not surprising, as these features are more likely to lead to a genetic consultation but may also lead to the high diagnostic rate. Further studies on the development of new features after diagnoses could aid in determination of natural history and prognosis of these conditions.

Previous studies have investigated single institutions, patients older than 1 month, patients only in neonatal intensive care units, or smaller cohorts of neonates in diagnostic rates of DES.[5,6] Additional, larger, diverse studies are needed to determine the cost-effectiveness of this testing, especially rapid DES in neonatal populations.

In summary, our data suggest that when assessing neonatal patients with a suspected genetic condition, clinical DES may be superior to traditional, comprehensive genetic-testing approaches for cases in which the clinical phenotype suggests one or more characterized syndromes. The option for rapid testing with results in 8 days gives the medical provider information that can potentially improve outcomes for this vulnerable population. In addition, continued contact with the reporting laboratory is important for continued care of the patient owing to new literature, development of new symptoms, and additional data leading to possible reclassifications. This work suggests that appropriate use of clinical DES may increase the rate of genetic diagnosis for neonatal patients.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

Z.P., K.D.F.H., L.S.M., V.S., T.C., K.B., J.M.H., M.T., and S.T. are employed by Ambry Genetics. Exome sequencing is among the commercially available tests. The other authors declare no conflict of interest.

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