Review Article

Challenges in the clinical understanding of genetic testing in birth defects and pediatric diseases

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Abstract: Advances in prenatal/neonatal genetic screening practices and next generation sequencing (NGS) technologies have made the detection of molecular causes of pediatric diseases increasingly more affordable, accessible, and rapid in return of results. In the past, families searching for answers often required diagnostic journeys leading to delays in targeted care and missed diagnoses. Non-invasive prenatal NGS is now used routinely in pregnancy, significantly altering the obstetric approach to early screening and evaluation of fetal anomalies. Similarly, exome sequencing (ES) and genome sequencing (GS) were once only available for research but are now used in patient care, impacting neonatal care and the field of neonatology as a whole. In this review we will summarize the growing body of literature on the role of ES/GS in prenatal/neonatal care, specifically in neonatal intensive care units (NICU), and the molecular diagnostic yield. Furthermore, we will discuss the impact of advances in genetic testing in prenatal/neonatal care and discuss challenges faced by clinicians and families. Clinical application of NGS has come with many challenges in counseling families on interpretation of diagnostic results and incidental findings, as well as re-interpretation of prior genetic test results. How genetic results may influence medical decision-making is highly nuanced and needs further study. The ethics of parental consent and disclosure of genetic conditions with limited therapeutic options continue to be debated in the medical genetics community. While these questions remain unanswered, the benefits of a standardized approach to genetic testing in the NICU will be highlighted by two case vignettes.

Keywords: Congenital disorder; genetic testing; neonatal intensive care unit (NICU); non-invasive prenatal testing (NIPT); prenatal counseling

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Introduction

The detection of genetic syndromes historically occurred after the birth of a child when certain phenotypic characteristics were recognized by the clinician. Many genetic conditions with mild or unapparent phenotypes in the newborn period would go unrecognized until later in life. Advances in ultrasonographic and genetic technology, namely next generation sequencing (NGS), have improved the detection of birth defects and permit earlier genetic diagnosis in the prenatal and neonatal period (1,2). Recommendations for genetic testing by the American College of Obstetrics and Gynecology (ACOG) and the American College of Medical Genetics and Genomics (ACMG) provide screening and diagnostic guidance in the prenatal and newborn period (3-5). Risk assessment is based on several factors, including maternal characteristics (e.g., advanced maternal age), carrier screening, family history, and the presence of fetal anomalies. First-line diagnostic testing during pregnancy usually includes karyotype and/or chromosomal microarray (CMA). Disorders not caused by chromosomal abnormalities or copy number variants, specifically single-gene disorders, are missed by these modalities.

A growing number of studies have investigated the yield and utility of whole exome sequencing (ES) and genome sequencing (GS) in prenatal and neonatal populations due to their increasing affordability and availability (6). ES provides information about single nucleotide variants and small insertion/deletions that are found in the coding regions of the genome, as well as information about some deletions/duplications or regions of excessive homozygosity in coding regions. GS generally provides information obtained from ES but with expanded coverage of genes, inclusion of noncoding regions such as introns, and increased sensitivity for structural variants. The diagnostic yield of ES/GS is dependent on the indication for testing (7). Given the concentration of conditions with potential genetic etiology in the neonatal intensive care unit (NICU), we will discuss the high diagnostic yields of neonatal ES/GS studies in NICU settings (*Table 1*). The purpose of this review is to discuss the impact of advances in genetic testing in prenatal and neonatal care in the NICU and present arising challenges for clinicians and the patients' families.

Prenatal screening strategies

The immediate purpose of prenatal genetic evaluation is to detect conditions that affect fetal, neonatal, and maternal health. Current ACOG, Society for Maternal-Fetal Medicine (SMFM), and ACMG guidelines recommend all pregnant individuals be offered genetic screening in the form of carrier screening, serum screening, and/or non-invasive prenatal testing (NIPT), and prenatal diagnostic testing regardless of risk (5,28-30). Previously, risk factors such as maternal age, presence of abnormality on fetal ultrasound, ethnicity, and family history were considered when recommending genetic screening and testing (3,28). Prenatal genetic screening assesses a pregnant person's risk of having a fetus affected by a defined set of genetic disorders, whereas prenatal diagnostic testing determines the presence or absence of a specific genetic condition in the fetus. All prenatal screening and testing options are voluntary and may be deferred by individuals who do not wish to obtain genetic information on their fetus. Parental carrier screening for some recessive conditions (e.g., cystic fibrosis, spinal muscular atrophy, and hemoglobinopathies) is also standard of care for reproductive genetic screening and has been reviewed elsewhere (31,32).

Screening tests include first and/or second trimester maternal serum analytes [pregnancy-associated plasma protein A (PAPP-A), beta human chorionic gonadotropin (hCG), alpha fetoprotein (AFP), estriol, inhibin A], first-trimester nuchal translucency ultrasound, and NIPT of cell-free fetal DNA (cfDNA). Single time point and combined screening test approaches are used, and the choice of screening test is influenced by multiple factors (3). In many settings NIPT has emerged as the preferred screening test and has rapidly changed the landscape of prenatal genetics (3,33). NIPT leverages NGS to assess cfDNA derived from the placenta circulating in the maternal bloodstream. The advantages of NIPT over the serum analyte-based approaches are earlier timing of testing (starting at 9–10 weeks of gestation) and higher sensitivity and specificity for common fetal aneuploidies (3). Although NIPT was first validated in high-risk patients, professional organizations have expanded their genetic screening recommendations to include the use of NIPT in all patients due to superior accuracy compared to maternal serum screening (3,28).

Since the detection of fetal cfDNA in maternal blood was first reported in 1997 (34), NIPT technology has expanded rapidly and multiple commercial tests are now available. All tests assess the risk of fetal Trisomy 21 (T21; Down syndrome), Trisomy 18 (T18; Edwards syndrome), Trisomy 13 (T13; Patau syndrome), and sex chromosome abnormalities such as 45,X (Turner syndrome) and 47,XXY (Klinefelter syndrome). The detection rate of NIPT is

Table 1 List of ES and GS studies involving NICU patients grouped by type of sequencing (8-27)

Author, year study name	Study type	Study interval	Inclusion criteria	Molecular diagnosis
D'Gama, 2022 (13)	ES, retrospective	Dec 2018 to Mar 2021	NICU admission; evaluation by genetics service	22/80 (28%)
Elliot, 2019 (16) RAPIDOMICS	ES, prospective	N/A	NICU admission; critical illness (seizures, metabolic abnormalities, neurologic symptoms, multiple congenital anomalies, physiologic disturbances)	18/25 (72%)
Kernohan, 2018 (10)	ES, re-analysis	2014 to 2015	NICU admission; undiagnosed medical condition (congenital malformation, dysmorphic features, growth abnormalities, neurological impairment), evaluation by genetics service	5/12 (42%)
Smith, 2020 (8)	ES, retrospective	Dec 2011 to Jun 2017	Age <1 year; ICU admission; evaluation by genetics service	101/368 (27%)
Swaggart, 2019 (9)	ES, retrospective	Jan 2013 to Dec 2014	NICU admission; followed via EMR until 2 years old	2/2 (100%)
van der Sluijs, 2019 (11)	ES, retrospective	Sep 2014 to Sep 2016	NICU/PICU admission; isolated cardiac anomaly; multiple congenital anomalies; delayed development	7/31 (22%)
Wells, 2022 (15)	ES, prospective	Apr 2019 to Apr 2021	Age <1 year; NICU/PICU admission; suspected genetic condition; trio samples available	7/15 (47%)
Williamson, 2021 (14)	ES, prospective	Oct 2019 to Sep 2020	NICU/PICU admission; critical illness; suspicion for a monogenic disorder; referral for testing by provider	7/8 (88%)
Yang, 2022 (12) China Neonatal Genomes Project	ES, prospective	Aug 2016 to Aug 2018	NICU admission; medical condition (e.g., anomalies of CNS, CV, GI/GU, skeletal; metabolic disorder; suspected immunodeficiency; hematologic abnormality)	284/2,303 (12%)
Meng, 2017 (17)	ES/GS, retrospective	Dec 2011 to Jan 2017	Age <100 days; hospital admission; clinical exome sequencing	102/278 (37%)
Suzuki, 2022 (20)	ES/GS, prospective	Apr 2019 to Mar 2021	Age <6 months; critical illness; congenital medical problems without diagnosis	41/85 (48%)
Wang, 2021 (18) China Neonatal Genomes Project	ES/GS, prospective	Dec 2016 to Dec 2019	Age <28 days; NICU or neonatal ward admission; at least two congenital anomalies	161/588 (27%)
Wu, 2021 (19) China Neonatal Genomes Project	ES/GS, prospective	Apr 2019 to Dec 2019	Age <13 months; NICU or neonatal ward admission; critical illness; medical condition (e.g., CNS anomaly, complex CHD, metabolic disorder, suspected immunodeficiency, multiple congenital anomalies)	74/202 (37%)
Bowling, 2022 (26) SouthSeq	GS, prospective	Feb 2018 to Jul 2020	Age <12 months, ICU admission, suspected genetic disorder	109/367 (30%)
Clark, 2019 (23)	GS, retrospective/ prospective	Jul 2016 to Sep 2018	Consecutive symptomatic children with genome sequencing	101/101 (100%)
Denommé-Pichon, 2022 (27) FASTGENOMICS	GS, prospective	Dec 2018 to Feb 2020	NICU/PICU admission, suspected genetic condition, trio sample availability	18/37 (49%)
Dimmock, 2021 (25) Project Baby Bear	GS, prospective	Nov 2018 to May 2020	Age <1 year old, within 1 week of hospitalization or abnormal response to therapy	74/184 (40%)

Table 1 (*continued*)

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Author (year) study name	Type	Study interval	Inclusion criteria	Molecular diagnosis
Lunke, 2020 (24) Australian Genomics Acute Care	GS, prospective	Mar 2018 to Feb 2019	NICU/PICU admission or likely to impact clinical management suspected monogenic conditions	55/108 (51%)
Palmquist, 2022 (22)	GS, retrospective	Oct 2015 to Oct 2022	NICU/PICU admission, referral for testing by provider, suspicion for a genetic condition	26/76 (34%)
Petrikin, 2018 (21)	GS, prospective	Oct 2014 to Jun 2016	Age <4 months; NICU/PICU admission; illness of unknown etiology	10/32 (31%)

ES, exome sequencing; GS, genome sequencing; N/A, not applicable; ICU, intensive care unit; NICU, neonatal intensive care units; EMR, electronic medical record; PICU, pediatric intensive care unit; CNS, central nervous system; CV, cardiovascular; GI, gastrointestinal; GU, genitourinary; CHD, coronary heart disease.

98.8% for T21, 98.83% for T18, and 92.85% for T13; the specificity is >99% for all three trisomies (35-38). Although the detection rate does not vary by maternal age group, the positive predictive value (PPV) is dependent on the population prevalence and can be further modified by the presence or absence of risk factors including fetal ultrasound findings (39).

Some companies also offer NIPT screening for microdeletion and duplication syndromes (e.g., 22q11.2 deletion, Prader-Willi, Angelman, Cri-du-chat), other aneuploidies (e.g., Trisomy 16, Trisomy 22), and genome-wide screening of large copy number changes. The use of NIPT to screen for these conditions is not routinely recommended because it has not been validated clinically and the false-positive rate is not well established (3,28). Clinical use of expanded NIPT platforms varies, and PPV for these conditions is increased in the presence of a fetal anomaly. Although expanded NIPT is not a replacement for diagnostic testing, the latter requires an invasive procedure with risk of complications. The authors have anecdotally encountered patients who elect expanded NIPT in the presence of a fetal anomaly as an alternative to invasive testing with appropriate counseling of the limitations and residual risks. Other applications of NIPT include the evaluation of fetal red blood cell antigen status (Rh, Kell) in patients with red blood cell isoimmunization (40,41) and detection of some single-gene disorders (42,43). Although these applications of NIPT are being used clinically in some settings, the accuracy and clinical utility are still being investigated.

Prenatal diagnostic testing

Invasive diagnostic prenatal testing directly evaluates placental tissue obtained from chorionic villus sampling (CVS) or fetal cells in the amniotic fluid collected by amniocentesis. CVS is performed between 10 and 13 weeks of gestation, while amniocentesis is typically performed in the second trimester starting at ~15 weeks of gestation after the amniotic and chorionic membranes have fused. Both procedures are available to all patients who desire diagnostic testing, though require referral to a maternal-fetal medicine specialist. Although prior prenatal screening is often performed, it is not required; diagnostic testing is commonly undertaken in the setting of elevated risk for genetic abnormalities due to advancing age, family history of a specific genetic disorder, or abnormal findings on ultrasound assessment of fetal anatomy (3).

Diagnostic genetic testing can evaluate a range of genetic changes, from abnormalities in chromosome number to single-gene variants. The specific genetic tests pursued depend on the indication for testing. In the setting of fetal structural anomalies, CMA is routinely offered (44). When a constellation of sonographic findings suggests a specific diagnosis or syndrome, targeted testing using fluorescence *in situ* hybridization (FISH) or gene panels can also be useful. The advantage of FISH is a faster turnaround time than karyotype for detection of specific chromosomal abnormalities, aiding in patient decision-making (45). When a single-gene disorder is suspected, single-gene testing or curated gene panels may facilitate targeted testing for genetic conditions such as *FGFR3* for achondroplasia or RASopathies panel testing for Noonan syndrome, respectively (46). Selection of an appropriate testing strategy relies heavily on multiple factors, including the patient's family and obstetrical history, suspected diagnoses, test performance, and ultrasound findings. The testing strategy is ideally determined in consultation with a clinical team that has expertise in fetal genetic diagnosis (47).

ES increases the yield of prenatal diagnostic testing and can be useful in situations where the karyotype and CMA are normal but a genetic diagnosis is strongly suspected (2,48). In a recent meta-analysis, the pooled diagnostic yield of ES in pregnancies with ultrasound anomalies was 31%, though the diagnostic rate is dependent on the indication for ES and other factors, including number of organ systems affected, the specific organ systems affected, and proband *vs.* trio sequencing (49). The classification of variants detected on CMA and ES is an ongoing challenge. Many detected variants are classified as "variants of uncertain significance" (VUS) and are subject to reclassification as new data emerge (1,2,50-52). If a variant was inherited from a parent, the clinical significance is often inferred based on the presence or absence of a phenotype in the affected parent, but incomplete penetrance and variable expressivity are important caveats. However, *de novo* VUS in the prenatal setting can be particularly challenging given uncertainties of the fetal phenotype.

Although prenatal ES is becoming more widely available in clinical care, there is still much debate on its use and implementation. Prenatal ES can present many challenges, including incidental findings in the parents and/or fetus and disclosure of secondary findings. The 2022 position statement from The International Society for Prenatal Diagnosis (ISPD) notes that routine use of prenatal ES cannot currently be supported due to insufficient validation data and the lack of knowledge of its benefits, risks, and limitations, especially in the absence of fetal anomalies (53). However, additional prospective studies for prenatal ES and GS are ongoing including Prenatal Genetic Diagnosis by Genomic Sequencing (PrenatalSEQ, ClinicalTrials.gov Identifier: NCT03936101), and utilization of these technologies is likely to increase as additional data become available and costs continue to decrease.

Advances in diagnostic genetic testing for newborns

Two NICU case vignettes are presented here to describe clinical scenarios that led to genetic testing, as well as the testing modalities that were used to reach a conclusive genetic diagnosis. These cases were selected to demonstrate the impact of discovering genetic conditions in the neonatal period for the patient and their families. All identifying information has been removed to protect the privacy of the patients and their families.

Case one: earlier identification of serious conditions

A male neonate was born at 40 weeks to a primigravid mother with complete prenatal care and no relevant past medical or family history. At birth, the newborn required routine resuscitation in the delivery room, but due to his labored work of breathing and oxygen requirement, he was brought to the NICU on 1 L/min nasal cannula with FiO_2 of 100%. He developed worsening hypoxic respiratory failure requiring escalation of respiratory support and was intubated for mechanical ventilation on day of life 1. Echocardiography revealed normal cardiac anatomy, a small patent ductus arteriosus with bidirectional low-velocity shunting, and significant flattening of the interventricular septum suggestive of elevated right ventricular systolic pressure. Inhaled nitric oxide was initiated on day of life 3 for presumed persistent pulmonary hypertension of the newborn, and the pulmonary hypertension team was consulted.

Findings on high-resolution chest CT were consistent with surfactant deficiency disease and pulmonary interstitial emphysema concerning for interstitial lung disease. Surfactant NGS panel that included *ABCA3*, *FOXF1*, *NKX2-1*, *SFTPB*, and *SFTPC* was sent (Fulgent Genetics, Temple City, CA). Genetic results revealed two likely pathogenic variants in *ABCA3* (c.614-1G>C and c.302T>C) consistent with surfactant deficiency. The patient required maximum ventilatory support and multiple agents to treat pulmonary hypertension including inhaled nitric oxide, sildenafil, ionotropic agents, high-dose steroids, and sedatives. The possible option of lung transplant was carefully considered by the family. They ultimately elected comfort-focused care, and the patient was extubated and died peacefully.

A high index of suspicion led the medical team and subspecialist to send a targeted genetic panel that provided a diagnosis and prognosis for the family. Parental testing confirmed that each parent was a carrier for one of the two likely pathogenic variants in *ABCA3*. This genetic information is critically important for future family planning. Additionally, the diagnosis provided information on tailored therapeutic options and prognosis, leading the parents to make a decision aligned with their goals to minimize painful procedures for their child. This case vignette exemplifies how genetic diagnosis can enable family-centered decision-making in the NICU setting.

Case two: whole GS and familial diagnoses

A male infant was delivered at 32 weeks due to preterm labor in the setting of placenta previa and recurrent episodes of vaginal bleeding. The mother had completed a course of antenatal corticosteroids prior to delivery. After birth, the neonate required immediate intubation in the delivery room. Following NICU admission, he required escalating respiratory support due to bilateral pleural effusions. Thoracentesis was performed and pleural studies confirmed the suspected diagnosis of congenital chylothorax. Other pertinent exam findings were macrocephaly, distinctive facial features, anasarca, and hypotonia. A wide array of suspected genetic conditions was considered, and rapid GS of the patient and parents (trio) were sent to Baylor Genetics, Houston, Texas. Data analysis and interpretation were performed by the Baylor Genetics analytics pipeline. Trinucleotide repeat calling was performed using the Illumina Manta Structural Variant Caller. The repeat number was confirmed by Southern blot. Genetic results confirmed congenital myotonic dystrophy type 1 (autosomal dominant), evident in a heterozygous pathogenic cytosine-thymine-guanin (CTG) repeat expansion of approximately 2,450 repeats in the 3' non-coding region of *DMPK* inherited from the mother (paternal testing was confirmed negative). The mother had a shorter CTG repeat expansion and a milder phenotype of a noticeably weak handshake. Her only other child, an older half sibling to the patient, has global developmental delay and was reportedly diagnosed with hypoxic-ischemic encephalopathy at birth but is now suspected to also have the genetic diagnosis and will receive testing in the near future.

Congenital chylothorax is not listed as a clinical feature of congenital myotonic dystrophy in Online Mendelian Inheritance in Man (OMIM.org). However, rare case reports and case series of pleural effusions and congenital chylothorax have been reported in congenital myotonic dystrophy (54-57). A number of genetic conditions were suspected by the medical genetics team including copy number variants and single gene disorders. Rather than selecting the typical first- and second-tier genetic test of CMA or gene panels, the medical geneticist favored rapid GS for this patient due to the broad differential diagnosis. While the high cost of ES and GS has limited their utility in clinical practice in the past, testing continues to become more affordable and may be considered cost-effective compared to other genetic tests (58,59). Additionally, ES and GS can take several weeks for results to return but may avoid the diagnostic journey families of children with rare and undiagnosed conditions often encounter in clinical practice. Certainly, in the case of this patient, confirmation of a genetic diagnosis was relatively quick and cost-effective compared to the usual, stepwise testing approach. The diagnosis also revealed a previously undiagnosed condition in other family members.

Genetic testing can be an important component of care provided in a NICU. All newborns receive screening by dried blood spot for a discrete list of early-onset disorders (varies by state), with DNA testing as primary- or second-tier confirmatory testing for the following conditions: cystic fibrosis, hemoglobinopathies, medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, severe combined immunodeficiency (SCID), and more recently, *SMN1*-related spinal muscular atrophy (SMA) (60). Expanding to universal genomic newborn screening is under investigation on the feasibility, clinical utility, public health benefits *vs.* costs, and ethical, legal, and social implications (61). Genomic newborn screening will not be discussed in this review as it is currently not used in the clinical setting, but has been addressed by others including a systemic review by Downie *et al.* (61).

Postnatal testing modalities are the same as those used prenatally and include karyotype, FISH, CMA, and gene sequencing. Adoption of clinical ES/GS varies by center, partially due to differences in pediatric genetics providers and genetic counselors. For example, at Boston Children's Hospital, rapid ES was available via a research study from March 2017 to November 2018 and then became part of routine clinical care (13). At the authors' respective institutions, rapid ES/GS are now options in clinical use. At both centers, to qualify for ES/GS, the primary NICU team identifies a patient with a suspected genetic disorder and consults the genetics team, and the teams jointly decide whether to request ES/GS. All ES/GS requests in the hospital are reviewed by a hospital committee. Results are reported approximately 14 days after sample submission.

ES/GS have become prevalent in the past decade and both have demonstrated varying diagnostic yields largely dependent on testing criteria (*Table 1*). Studies of ES/GS that include NICU patients have generally indicated high rates of molecular diagnoses, ranging from 12% to 51% in studies of at least 100 infants (8,12,17-19,24-26). In the first 28 months of routine use of rapid ES at Boston Children's Hospital, 22/80 (28%) patients who had ES performed received a molecular diagnosis from the results (13).

Initial ES/GS studies were retrospective and did not consistently demonstrate improved diagnostic yield over clinical genetic evaluation that did not include ES/GS (8). However, newer prospective studies suggest improved diagnostic yield, decreased time to diagnosis, and impact on patient care. Potential sources of variance in diagnostic rates include differences in variant assessment pipelines between companies and sequencing platforms, differences in inclusion/exclusion criteria, and use of ES *vs.* GS. Two smaller studies that included only patients with critical illness and/or suspicion for a monogenic disease reported diagnostic rates greater than 70% (14,16). Certain patient characteristics, such as neurological signs or symptoms, or multiple congenital anomalies, are associated with increased rates of molecular diagnosis. Several multicenter studies in different countries have demonstrated the reproducible benefit of ES/GS in children with explicit phenotypic criteria for diagnosis and clinical decision making (24,62,63).

ES does not detect variants outside of capture regions, such as noncoding variants, and many tandem repeat elements (64,65). Thus, genetic conditions such as congenital myotonic dystrophy described in the second NICU case vignette above are not well detected by most ES analyses. Current exome analytical pipelines are also less sensitive for small structural variants such as deletions involving only one exon. GS analysis can be optimized to detect many tandem repeat and small structural variants (66). Furthermore, methylation state is not detected by ES, so some causes of Beckwith-Wiedemann and Prader-Willi cannot to be diagnosed by ES (67). ES is able to detect uniparental disomy and deletions which could be an alternative cause of imprinting disorders. Therefore, many centers consider targeted testing for triplet repeat disorders and methylation disorders and rapid chromosomal microarray in conjunction with ES for internal exon deletions (62).

Ongoing challenges of prenatal and neonatal genetic testing

As the landscape of prenatal and neonatal genetic testing evolves, we will continue to encounter new challenges that require thoughtful strategies to maximize benefits and minimize harms while centering the patient's goals and values. For example, concerns over newborn screening dried blood spot storage and utilization and possible universal genomic newborn screening are related to an insufficient informed consent process in current newborn screening programs, in contrast to consent obtained for genetic testing performed for a suspected genetic condition (68,69). For the latter, three major challenge areas are: (I) patient understanding of testing, (II) detection of secondary findings, incidental findings, and reinterpretations; (III) impact on clinical management.

The decision to pursue genetic screening or testing should occur after a comprehensive discussion about options and with informed consent, especially given the rapid advances in technology that have added complexity and nuance to prenatal and neonatal genetics. In the prenatal setting, consultation with a genetic counselor or educational resources should be offered to all patients as a standard component of prenatal care (47,70,71). If a genetic diagnosis is suspected and confirmed, genetic counselors can provide information on prognosis and recurrence risk for future family planning. Most pediatric rare diseases are due to pathogenic *de novo* genetic variants in autosomal dominant disorders or *de novo* aneuploidy, both with extremely low risk of recurrence (72). In cases of autosomal recessive or inherited autosomal dominant or X-linked disorders, the recurrence risks are much higher. It is vital that post-test counseling of abnormal results include discussion on the specific recurrence risks and options for family planning, including prenatal diagnosis and preimplantation genetic testing. While these scenarios have long been a part of the genetic counselor's experience in single gene disorders, the landscape has changed significantly with the expansion of ES/GS (73).

Many of the newer challenges result from a lack of clear communication and education about the purpose and limitations of different tests. A recent study reported that among patients who had undergone NIPT, one in five were unaware that the screening was done and nearly one-third did not recall discussing NIPT with their prenatal provider (74). Having discussions up front about the limitations of prenatal genetic screening and testing can avoid misunderstandings and the negative repercussions of missed, delayed, and incorrect diagnoses. Examples include:

- False reassurance. Patients who are unaware that screening tests estimate risk for a limited number of genetic conditions may assume that a negative result excludes the possibility of a genetic condition (75).
- Misinterpretation of screen positive results. Patients may not understand the differences between a screening test and diagnostic test not the possibility of a false positive result. Inadequate counseling in this scenario can lead parents to

believe that a fetal genetic diagnosis has been established despite a lack of confirmatory testing (76,77). All patients should be offered invasive diagnostic testing to confirm genetic conditions suggested by prenatal screening (3).

- ❖ Failure to recognize the biologic reasons for discordant NIPT results. Discordance between NIPT results and fetal phenotype or genotype occurs because NIPT detects cfDNA in the maternal circulation which comes from many sources in addition to the placenta. For instance, discordant fetal sex (NIPT-predicted male fetus in the case of a female fetus) can occur in the setting of a vanished male twin or a history of maternal organ transplant (e.g., kidney from a male donor) (78). A sex chromosome discrepancy may also suggest a disorder of sexual development (79). NIPT showing an increased risk of aneuploidy in a euploid fetus can be the result of confined placental mosaicism, maternal mosaicism, or occult maternal malignancy (80).

In addition, counseling must address the potential detection of secondary and incidental findings. As alluded to above, NIPT and trio ES/GS have the potential to reveal genetic results that parents are not intentionally seeking, including the presence of genetic or other health conditions in the parents. These incidental findings can be surprising to parents so adequate pretest counseling is essential. In the Baby Beyond Hearing project, families were offered exome sequencing for congenital deafness in their child, and in addition to a genetic diagnosis for deafness, they were given the choice to also receive additional findings of childhood-onset diseases with and without medical actionability (81). Families with infants less than 3 months of age were more likely to decline receiving additional findings. Family values and circumstances are important factors to consider in the discovery and reporting of secondary and incidental findings. Lastly, in circumstances of consent by proxy, ethical considerations become more complex when considering the emotional and economic impact on a child from a disclosed genetic diagnosis obtained before they were of the age to consent.

Pretest counseling should also include the possibility of not finding a diagnosis or of an uncertain diagnosis in the case of VUS with the potential responsibility of reinterpretation (53). In the prenatal setting, patients may receive an indeterminate or no-call result from NIPT due to low fetal fraction (the percentage of total maternal plasma cfDNA that is of fetoplacental origin) (82). These patients are at an increased risk of aneuploidy and require additional investigation (36). VUS can be difficult to interpret for non-geneticists and confusing for families as interpretation is based on the premise that the clinical significance is unknown at the time of genetic testing, but may evolve over time. As genetic testing becomes increasingly generalized, genotype-phenotype correlations will become better-defined, and variants that previously had unknown significance may later be recategorized as pathogenic. There is no standardized process for reinterpretation of VUS, and recontacting families remains a topic of debate in the medical genetics community (83). The duty to reinterpret is at odds with the potential ethical dilemma of recontacting patients without their consent. Importantly, the benefits of reinterpretation may not be evenly distributed, exacerbating health disparities (84).

Broader prenatal and postnatal genetic testing has led to earlier detection of genetic conditions associated with high rates of neonatal mortality. In the field of neonatology, this experience can be best characterized in the care of patients with T13 and T18 and the ethical dilemmas encountered at this crossroads of neonatal care and genetics. Advances in neonatal intensive care and medical interventions have led to improved survival of those children and therefore gaps exist between historical outcomes data and current patient experiences (85,86). Patients with T13 and T18 have severely shortened life expectancies, however increased testing has revealed milder phenotypes due to mosaicism with longer survival rates. Decisions by families to pursue interventions like cardiac surgery have also extended survival for children with full T13 or T18 (87). These circumstances are likely to result in shifting attitudes and expectations among families and the medical community. The timing of genetic diagnosis can also influence the counseling by providers and decisions made by the family. Families with a prenatal diagnosis of T13 or T18 are generally recommended comfort care by prenatal providers (87). Not surprisingly, a prenatal diagnosis is the most important factor associated with mortality. In contrast, children diagnosed postnatally are provided full intervention until families receive the T13 or T18 diagnosis, followed by some families withdrawing invasive care. Similarly, what parents perceived as palliative care may differ based on the timing of diagnosis. For prenatal diagnoses, palliative care usually involves minimal to no interventions to extend life, while for postnatal diagnoses, palliative care could include medical and surgical interventions to optimize quality of life including tube feedings, non-invasive respiratory

support, and "palliative" cardiac surgery (e.g., ventricular septal defect closure) (88). Neonatologists are challenged with providing sensitive prenatal counseling and postnatal care based on shared decision making with families and identified goals of care (89).

Conclusions

As the reviewed ES/GS studies and our two case vignettes highlight, clinical application of NGS has provided many opportunities to improve prenatal and neonatal care. NIPT and ES/GS have proven utility in selected circumstances, but the lack of targeted interventions also points to important future directions to enhance the role of molecular diagnoses in neonatal care. Many genetics variants have been identified in children that have not previously been reported to be associated with disease, but also have not been observed in large cohorts of adults without known genetic disease. Future re-analysis of genetic data can result in higher rates of molecular diagnoses using updated annotations of genes and variants. Given the high yield of ES/GS among NICU patients, as well as the demonstration of significant clinical impact of genetic results, ES/GS availability should be increased with attention to patient equity. This can be accelerated by the reduction in the cost of testing, improved representation of all genetic ancestry groups in reference cohorts, and increased access to medical professionals skilled in obtaining informed consent for genetic studies as well as the communication of results to families and providers. With these goals in mind, we can continue to improve equitable access to precision medicine in maternal and pediatric health.

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Footnote

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