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The Role of Genome Sequencing in Neonatal Intensive Care Units

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Abstract

Genetic diseases disrupt the functionality of an infant's genome during fetal–neonatal adaptation and represent a leading cause of neonatal and infant mortality in the United States. Due to disease acuity, gene locus and allelic heterogeneity, and overlapping and diverse clinical phenotypes, diagnostic genome sequencing in neonatal intensive care units has required the development of methods to shorten turnaround times and improve genomic interpretation. From 2012 to 2021, 31 clinical studies documented the diagnostic and clinical utility of first-tier rapid or ultrarapid whole-genome sequencing through cost-effective identification of pathogenic genomic variants that change medical management, suggest new therapeutic strategies, and refine prognoses. Genomic diagnosis also permits prediction of reproductive recurrence risk for parents and surviving probands. Using implementation science and quality improvement, deployment of a genomic learning healthcare system will contribute to a reduction of neonatal and infant mortality through the integration of genome sequencing into best-practice neonatal intensive care.

INTRODUCTION: THE UNIQUE GENOMICS OF NEONATOLOGY

The perinatal period, defined as 6 months before to 12 months after birth, is unique in terms of healthcare consequences across the lifetime. During this period, the functionality of a child's genome is tested by the biologic requirements of the fetal–neonatal transition and extrauterine physiologic adaptation. During pregnancy and the immediate postpartum period, many fetal physiologic processes are provided, or compensated for, by the maternal or placental genome. In addition, genome-encoded developmental regulation continues to activate and silence genes and gene pathways throughout the first months and years of life. For example, drug metabolism is quite different in newborns and older children due to lack of expression of the key metabolic enzyme cytochrome P450 2D6 (92). Finally, delivery of viable fetuses may occur at diverse postconception time points. Thus, in caring for newborns, neonatologists must consider age both as days since conception and as days since delivery.

Much remains to be learned about the factors that regulate the ontogeny of gene expression and silencing associated with neonatal and gestational ages (92). The genetic, genomic, and functional genomic regulation of fetal development and neonatal adaptation to the extrauterine environment is uniquely challenging in three ways. First, the timing of onset of monogenic disease is regulated by the continuum of causal gene expression in the fetus or newborn during the perinatal period, as suggested by examples of genetic disruption of liver and cerebral function (67, 88). Second, the timing of onset of monogenic disease is influenced by the continuum of compensatory effects of maternal or placental genes. For example, in the setting of primary neonatal immunodeficiency, maternal immunoglobulins may protect a newborn from infections for months (19, 96). Third, the adaptive physiologic changes that occur at delivery immediately expose latent genetic defects. Third-trimester echocardiography in fetuses with congenital heart disease, for example, can define cardiac anatomy but may not be able to predict neonatal cardiac adaptation to extrauterine life.

While we can now routinely decode and analyze a human genome in a day, the contributions of many genetic defects to disease penetrance, severity, onset, complications, response to treatment, and outcomes need to be defined to permit clinically actionable genomic results and remain under active investigation (40, 75). Similar to comparing a perfect space rocket at *t* minus 5 minutes to launch and *t* plus 5 minutes after launch, the phenotypically normal fetus may develop life-threatening genetic disease in the neonatal period whose treatment and prognosis may be difficult to predict based on fetal phenotype. For example, knowledge of the underlying genetics in developmental epileptic encephalopathies does not necessarily translate today into clinically actionable guidance for treatment or prognosis. While we have identified genetic variants associated with more than 7,000 genetic diseases, for a majority, we do not yet understand the encoded mechanisms of action.

Another challenge to the use of genomic information for sick infants is that different variants in a single gene may be associated with different disease phenotypes (76), and similar disease phenotypes can be associated with different genes (55). The former is particularly perplexing. For example, variants in a voltage-gated potassium channel gene, *KCNQ2*, map to both developmental and epileptic encephalopathy type 7 (OMIM 613720) and benign neonatal seizures type 1, also known as myokymia (OMIM 12100). The treatment and prognosis of these two disorders are quite different. Benign neonatal seizures type 1 presents with seizure onset at 2–8 days of life; seizures respond to first-line antiepileptic medications, such as phenobarbital, and most remit by 6 weeks. Developmental and epileptic encephalopathy type 7 has a similar age of seizure onset but requires much more aggressive antiepileptic therapy and is associated with developmental delay and intellectual disability.

In summary, while the greatest potential contribution of genome sequencing to human health is currently for newborns, the unique physiologic and genomic characteristics of newborns plus our incomplete understanding of encoded mechanisms of action, treatment, and prognosis present bottlenecks to achieving the full potential of timely genetic diagnosis for reducing fetal and neonatal morbidity and mortality. However, the importance of the contributions of genetic disease to neonatal and infant mortality has accelerated study and the integration of genome sequencing into neonatal intensive care units (NICUs) to improve infant outcomes.

NICU: neonatal intensive care unit

WES: whole-exome sequencing

WGS: whole-genome sequencing

rWES: rapid whole-exome sequencing

rWGS: rapid whole-genome sequencing

The Value of Genome Sequencing in Neonatal Intensive Care Units

Although many of the physiologically supportive clinical practices of neonatal intensive care help to improve the survival of critically ill infants with genetic diseases, many affected infants have unique disease mechanisms associated with pathogenic genomic variants that are rare or novel due to reduced reproductive fitness (49) and disrupt the function and/or anatomy of multiple organs (91). An evidence-based clinical practice guideline from the American College of Medical Genetics and Genomics supports the clinical utility and desirable effects of whole-exome sequencing (WES) and whole-genome sequencing (WGS) on active and long-term clinical management for pediatric patients under 1 year of age with 1 or more congenital anomalies (56). NICUs have recognized the critical need for diagnostic discovery of genomic causes of newborn diseases to individualize treatment, establish prognoses, predict reproductive recurrence risk, and improve infant outcomes (44, 107). Leveraging collaborations with geneticists, genetic counselors, obstetricians, genomicists, and bioinformaticians; advances in genome sequencing technology [especially reductions in turnaround time (75)]; computational strategies for identifying pathogenic variants (61); and reduced sequencing costs, NICUs have begun transitioning from a phenotype-first to a genotype-first approach for genetic diagnosis in critically ill infants by integrating trio rapid whole-exome sequencing (rWES) and rapid whole-genome sequencing (rWGS) into early diagnostic testing, which will permit faster diagnosis, more precise prognosis, and rapid identification of individualized treatment options (43, 75). In addition, genotype-first diagnosis provides families with estimates of reproductive recurrence risk for future pregnancies and for the surviving proband and siblings. To illustrate the diagnostic and therapeutic value of rWES and rWGS for NICU patients with congenital anomalies, we briefly discuss epileptic encephalopathy due to thiamine metabolism dysfunction syndrome as an example.

Epileptic Encephalopathy Due to Thiamine Metabolism Dysfunction Syndrome

Although infections, trauma, and environmental factors are associated with epileptic encephalopathies, genetic etiologies contribute significantly to their frequency and pathogenesis (5, 93). Due to purifying selection, genetic causes are heterogeneous. Discovery of a monogenic etiology in an infant with rWGS can inform precision therapy, as illustrated by a recently reported case (75). Briefly, a 5-week-old, previously healthy male infant of consanguineous parents whose reproductive history included a previous child who had expired with epileptic encephalopathy presented with epileptic encephalopathy. rWGS (within 16.5 h) revealed homozygous, known pathogenic (ClinVar VCV000533549 0.2) frameshift variants in *SLC19A3*, which encodes a thiamine transporter and is known to be a monogenic cause of thiamine metabolism dysfunction syndrome 2 (OMIM 607483)—a condition that, if untreated, leads to rapid neurologic deterioration and death (52). Based on these findings, treatment with thiamine and biotin was initiated, with almost immediate resolution of seizures, and at 7 months of age, the infant was reportedly thriving. This patient illustrates the importance of integrating rWGS for critically ill infants with congenital anomalies such as epileptic encephalopathies, metabolic disorders, and structural birth defects.

INTEGRATING GENOME SEQUENCING IN NEONATAL INTENSIVE CARE UNITS

The opportunity for genetics and genomics to reduce neonatal morbidity and mortality has grown over the last two decades after NICUs developed life-sustaining obstetric, surgical, and medical strategies for infants born at the edge of viability, with life-limiting congenital anomalies, neonatal infections, and severe intrapartum insults. In the early twentieth century, newborns were delivered at home and survived if they were able to establish respiration, maintain oral intake, and avoid infection. Between 1930 and 1950, deliveries shifted from homes to hospitals to gain access to maternal anesthesia and analgesia. Hospital staff and families recognized that the survival of premature infants could be significantly improved by providing thermoregulation, oxygen, hand hygiene, and more intensive physiologic monitoring. This recognition prompted the establishment of the first NICUs in the 1960s.

With increased neonatal specialization and favorable reimbursement, the number of NICUs has rapidly expanded over the past three decades, to approximately 800 units in the United States (30). Concurrent with broad deployment and refinement of neonatal intensive care, the neonatal mortality rate (death within the first 28 days of life) has fallen from 18.7 (1960) to 3.8 (2018) per 1,000 live births. This survival improvement is attributable to the collaboration of multidisciplinary teams of nurses; physicians (neonatologists, obstetricians, anesthesiologists, and pediatric subspecialists); respiratory, occupational, and physical therapists; social workers; pharmacists; and clinical investigators focused on the unique physiologic challenges of extrauterine adaptation. These teams have developed, implemented, and deployed quality improvement frameworks with specific care practices, interventions, and quantitative clinical guidelines that are specific to newborns' unique transitional physiology and disease susceptibilities. For example, interventions to improve pulmonary outcomes for premature infants (e.g., antenatal glucocorticoid administration, surfactant replacement therapy, and less invasive ventilation strategies) combined to reduce a previously common cause of neonatal mortality in preterm infants, respiratory distress syndrome, from 20% (1980) to 2% (2019) of infant deaths.

As a consequence, the most frequent causes of infant deaths in the United States based on birth certificate data are now genetic and include congenital malformations, deformations, and chromosomal abnormalities, which collectively accounted for 20.6% of 20,921 total infant deaths in the United States in 2019 (1, 35). Many, but not all, of these deaths are caused by single-locus genetic diseases. Approximately 10% of the US annual birth cohort (~400,000 newborns) are now admitted to NICUs, costing at least $26 billion (in 2007 dollars) and accounting for up to 50% of the total US pediatric health care expenditure (33). In addition, 10–25% of critically ill infants in NICUs are estimated to have an undiagnosed single-locus disorder that may be missed due to the nonspecific presentations of many genetic diseases in the newborn period and to limitations in reimbursement for comprehensive inpatient genetic testing (34, 58, 106, 107).

Prior to the availability of WES/WGS in the NICU, genetic diagnosis in critically ill newborn infants relied on both a prenatal and postnatal phenotype-first approach. Strategies to assess fetal phenotype, including ultrasound, maternal biochemical testing, and fetal magnetic resonance imaging, provide fetal characteristics that may not be specific to individual genetic diagnoses (34). Historically, fetal and neonatal genetic testing was highly selective and employed chromosomal analysis (karyotyping, fluorescence in situ hybridization, and chromosomal microarray analysis) and Sanger sequencing of exons of specific candidate genes. Samples for fetal DNA extraction included chorionic villi, amniocentesis fluid, fetal umbilical blood, or maternal cell-free DNA. Diagnoses were limited to aneuploidy, large copy number variants, and recurrent single-locus genetic diseases with pathognomonic features (10, 34, 41, 47, 50, 105). Until very recently, there

was considerable uncertainty in estimates of the incidence or types of genetic diseases among infants and children hospitalized for critical illness (54, 59). Furthermore, the etiology of common presentations was frequently regarded as polygenic, influenced heavily by the common disease–common variant hypothesis and results of genome-wide association studies. We now know that infant-onset conditions such as epilepsy, developmental delay, intellectual disability, hearing loss, and inflammatory bowel disease are actually each reflective of at least 1,000 separate single-locus genetic diseases. In addition to a lack of comprehensive testing modalities, patenting of disease genes created barriers to diagnostic testing (38, 46).

Comprehensive genetic testing first became available with the advent of next-generation sequencing in 2005 (57). The first individual human genomes were sequenced from 2007 to 2009 at a cost of at least $1 million (42, 53). This uniquely exciting time in human genetics generated multiple novel realizations. For example, comprehensive sequencing revealed that genomes contain many more variants than suspected (4–6.5 million per individual). Disease gene discovery was less computationally challenging when trio genome sequencing (parents plus proband) replaced the traditional approach of linkage analysis and positional cloning. An exciting development in 2009 and 2010 was the use of WES and gene panel exome sequencing (71). The exome refers to the collection of all exons of approximately 20,000 protein-coding genes and makes up approximately 2% of the genome. While exomes must be sequenced much more deeply than genomes (90-fold versus 30-fold) for comprehensive coverage (because of coverage skewing), they decreased the cost of genome sequencing approximately 10-fold (46).

The era of the $1,000 research genome led to many discoveries. Almost overnight, human genetics went from being descriptive to being quantitative. Advances in bioinformatics facilitated the development of high-performance computing strategies necessary for genomic data management and interpretation. We realized that human genomes had many more de novo variants than previously recognized (~80 per genome), and that the resultant dominant disorders contributed the majority of single-locus genetic disease diagnoses in outbred populations (48). The success of positional cloning for identifying recessive conditions led to the erroneous conclusion that they were much more common than dominant disorders.

The first use of WES to diagnose genetic diseases was in 2009 (14, 70), and the first gene panel sequencing test for diagnosis and carrier testing was in 2011 (6). Two landmark papers at that time showed the ability of WGS- or WES-based diagnosis to dramatically change childhood outcomes (3, 108). Many physician–scientists who had trained in adult internal medicine, believing it to be the most rigorous clinical discipline [as, for example, one of us did (S.F.K.)], suddenly realized that the next decade would be dominated by pediatric molecular discoveries! We realized, to our great chagrin, that 27% of variants in our databases had been misclassified as pathogenic (6). As a community, we had been looking under the lamppost for our lost diagnostic keys and thereby overinterpreting pathogenicity.

Diagnostic rWGS of infants with suspected genetic diseases in NICUs became possible in 2012 (83). Before that time, return of results from WES/WGS took several months, limiting diagnostic use to outpatients (**Figure 1**). Faster sequencing and bioinformatics, together with semiautomated diagnostic interpretation, decreased the time to genome sequencing result to 50 h.

In the past 10 years, the turnaround time, scalability, cost, and diagnostic yield of rWGS have continued to improve iteratively (**Figure 1**). The minimum turnaround time is now 13.5 h (75). WGS is scalable to entire populations (29), and the minimum cost of a research genome is now approximately $500 (69). Diagnostic capacity has steadily expanded to include disorders of the mitochondrial genome, structural and copy number variants, simple sequence repeat expansions, imprinting disorders, and loci featuring pseudogenes. Sensitivity and specificity for single-nucleotide variants now exceed 99.5% (74). Diagnostic rWES and rapid diagnostic gene panel

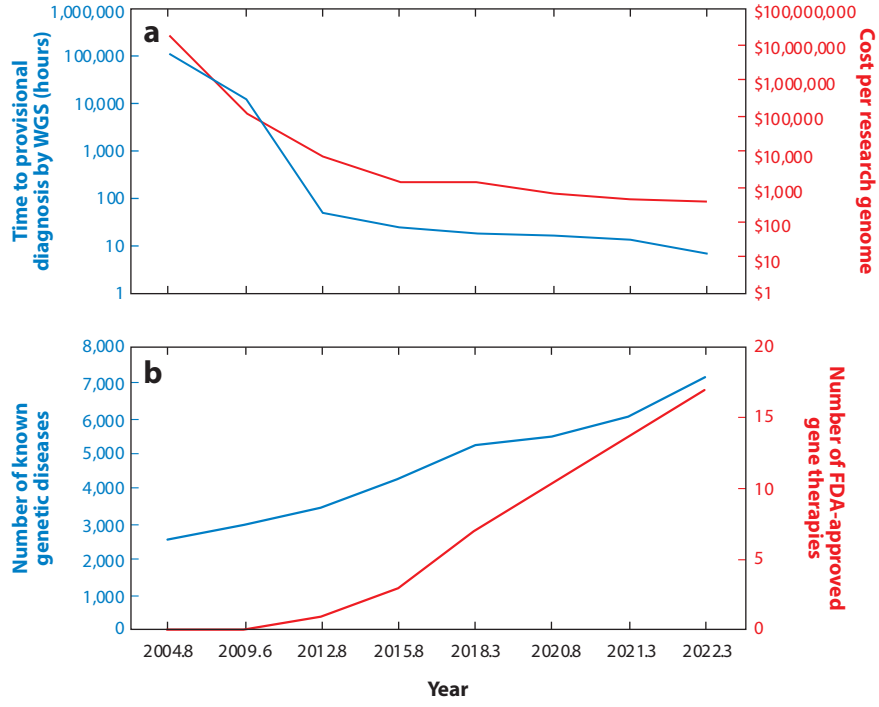

Figure 1

The current inflection point in precision neonatology for single-locus genetic diseases. (*a*) The cost of research-grade WGS (*red line*) and the time to result for diagnostic WGS (*blue line*) have been decreasing over the past 10 years. (*b*) The number of known genetic diseases (*blue line*) has increased dramatically since the advent of next-generation sequencing. The number of approved gene therapies for childhood-onset genetic diseases (*red line*) has also rapidly increased. Abbreviation: WGS, whole-genome sequencing.

exome sequencing became available shortly after rapid genome sequencing. They remain less expensive than genome sequencing ($2,000–$2,500 for rapid diagnostic gene panels, $4,000–$5,000 for rWES, and $8,000–$10,000 for rWGS). They cannot, however, be performed as rapidly as genome sequencing, because they require exon enrichment and amplification steps. While their diagnostic yield has been similar to that of genome sequencing, as our ability to detect pathogenic, nonexonic variants improves, WGS will inevitably become superior.

RAPID GENOME SEQUENCING TECHNOLOGY

The technology, infrastructure, and computational strategies that enable NICU rWGS have become highly standardized (**Figure 2**). First, parental consent is sought. This consent is necessary because of the possibility of harm for the infant. In the United States, most potential harm was mitigated by the Genetic Information Nondiscrimination Act of 2008. The scope of testing must also be determined with parental consent. Parent–infant trio testing is faster and has a higher potential diagnostic yield than singleton testing (17). Some parents, however, such as US Armed Forces service members, are not protected by the Genetic Information Nondiscrimination Act. Parents must also decide whether they wish incidental findings—genomic variants that are not related to the infant's current illness but could have significant consequences for future health—to be returned (65, 66). Finally, there is the option of rapid and ultrarapid testing. The latter is

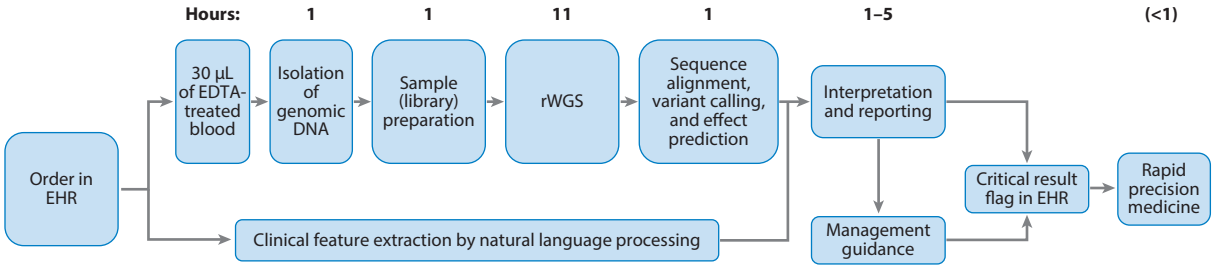

Figure 2

Steps and minimum times for rapid genetic disease diagnosis by WGS and implementation of precision neonatology. Abbreviations: EDTA, ethylenediaminetetraacetic acid; EHR, electronic health record; rWGS, rapid whole-genome sequencing; WGS, whole-genome sequencing.

reserved for the subset of NICU infants in whom time to result is critical, either because of the severity of their illness or because of time constraints of medical decision-making or significant interventions (such as starting extracorporeal membrane oxygenation).

Diagnostic genome sequencing requires two inputs. First, the clinical features of the child's illness are extracted from the medical record. This extraction is often performed computationally by natural language processing. The observed clinical features, typically as Human Phenotype Ontology terms, are then compared computationally with the expected clinical features of all known genetic diseases to create a quantitatively prioritized differential diagnosis list. Alternatively, the clinical features can be used to select from a set of virtual gene panels. The second input is the genome sequence from DNA extracted from 30 µL of peripheral blood. The DNA is then prepared for sequencing, a process called library preparation, which involves random fragmentation into approximately 500 nucleotide pieces and the attachment of short DNA probes on either end. Genome sequencing is then performed. Exome and gene panel sequencing require two additional steps: enrichment of exons and amplification of the remaining material.

Sample preparation, from the initial blood sampling to the start of sequencing, takes from 2 h to 2 days. The longer preparation time is required for exome and panel sequencing. Sequencing is almost always by synthesis, resulting in a pair of 100–150-nucleotide sequences (reads) from either end of each fragment. Genomes are sequenced to at least 30-fold coverage (~100 Gb of DNA sequence), while exomes are sequenced to at least 90-fold coverage (~10 Gb). Sequencing takes 11 h to 2 days, depending on instrument settings, and is performed from single samples all the way to 50 samples per run.

The remaining steps are computational and performed either in a cloud environment or on local high-performance computing instruments. First, the nucleotide of each position on each read is determined, together with a quality score. In general, the quality must be better than 1 error in 1,000 (quality score of >30). Second, each pair of sequences is mapped to the corresponding unique region of the genome (alignment), and a mapping quality score is determined. Third, the aggregate sequence at each position is determined based on the consensus of approximately 30 reads in genome sequencing. This involves determining the DNA sequence, zygosity, and copy number. All nucleotides and regions that differ from the reference genome are identified (variant calling). A typical genome will have at least 3.5–5 million single-nucleotide substitutions, 750,000–1 million insertions or deletions that are 1–50 nucleotides in size, and 20,000 structural or copy number variants that range in size from 50 nucleotides to entire chromosomes (**Table 1**).

Next, variants are mapped to genes, genes to diseases, and diseases to the differential diagnosis calculated based on the infant's clinical features. To diagnose a genetic disease, the search space

Table 1 Comparison of the median analytic performance of rWES ($n = 95$) and rWGS ($n = 118$)

	Coding nucleotides with ≥10× coverage	Total variants	SNVs	Indels	Coding variants	Rare variants (MAF <1%)	Variants in OMIM disease genes	Missense variants	Nonsense variants	Altered canonical splice sites	Frameshift indels	Disrupted start codons
rWES	94.5%	38,901	35,465	3,401	23,421	2,703	670	558	14	15	46	3
rWGS	98%	4,669,310	3,792,213	881,699	26,080	240,648	48,231	687	16	82	85	4
Fold difference	1.04	121	107	258	1.12	85	69	1.3	1.3	5.4	1.8	1.3
p value[a]	NA	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0

Data are from Reference 43. Abbreviations: indel, insertion or deletion; MAF, minor allele frequency; NA, not applicable; rWES, rapid whole-exome sequencing; rWGS, rapid whole-genome sequencing; SNV, single-nucleotide variant.
[a]Wilcoxon signed-rank adjusted *p* values.

is almost always limited to the identified genes. Pathogenicity prediction algorithms are used to predict the effect of each variant on gene or protein function. Approximately 99% of variants are predicted to not affect function (**Table 1**). OMIM lists 7,036 genetic diseases that map to 4,545 genes (39). Due to the low frequencies of neonatal genetic diseases associated with purifying selection pressure, all variants that are common in populations are discarded. With a few exceptions, the cutoff is typically a 1% minor allele frequency, removing 95% of variants (**Table 1**). These steps generate a short list of variants that are evaluated one by one based on a large number of additional considerations, such as diplotype zygosity, allele frequency, computational pathogenicity prediction, and de novo occurrence. Each variant is compared with several reference databases of disease-causing and non-disease-causing variants. Depending on the patient's age, location (such as the NICU), and clinical features, a genetic disease diagnosis is made in 10–50% of cases. In 5% of cases, two genetic diseases coexist. In addition to findings that are considered diagnostic, in 20–35% of cases, variants of uncertain significance (VUSs) in genes associated with the phenotypic characteristics of the child's presentation are identified. These are often called VUS-suspicious and are typically also reported. In 5–10% of cases, there will be incidental findings (diagnostic findings that are considered unrelated to the child's current illness).

This process—genome interpretation—can be fully automated, taking 5–15 min, with retention of approximately 80% sensitivity and 50% specificity, values that are currently insufficient to permit autonomous performance (20). Expert manual interpretation by specialist staff, including physicians and molecular laboratory directors, usually takes 1 h to 1 day. Typically, results likely to change management in a highly beneficial manner are immediately returned verbally as a provisional report. Approximately one-third of results undergo orthogonal confirmatory testing to, for example, verify that two variants in a recessive condition are in *trans*, confirm de novo status when parental genomic sequences are not available, or exclude the possibility that variants are false positives.

DIAGNOSTIC AND CLINICAL UTILITY OF RAPID GENOME SEQUENCING

VUS: variant of uncertain significance

ICU: intensive care unit

The diagnostic yield of rapid genome sequencing in infants and children in intensive care units (ICUs) was evaluated in 31 clinical studies from 2012 to 2021 (**Table 2**). Most were cohort studies. Of the 31 studies, 16 evaluated rWES, 12 evaluated rWGS or ultrarapid WGS (urWGS), 2 evaluated both rWES and r/urWGS, and 1 evaluated both rWGS and rapid panel testing. While the inclusion criteria, clinical settings, and hospital systems varied, all of the studies evaluated the diagnostic yield in children in ICUs with suspected genetic diseases. Average turnaround time varied from 0.8 days to 60 days. The weighted average rate of genetic disease diagnosis was 36%

Table 2 Studies of the diagnostic performance, clinical utility, and change in outcome of rWES, rWGS, urWGS, and panel tests in seriously ill children in ICUs

Year(s)	Reference(s)	Study type	Test type(s)	Enrollment criteria	Size	Diagnostic rate	Change in management	Change in outcome	TAT (days)
2012	83	Cases	urWGS	Infants in a NICU with a suspected genetic disease	4	75%	NE	NE	2
2015	104	Cohort	rWGS	Infants <4 months old with a suspected actionable genetic disease	35	57%	31%	29%	23
2017	62	Cohort	rWES	Infants <100 days old with a suspected genetic disease	63	51%	37%	19%	13
	97	Cohort	rWGS	Infants in a NICU or PICU with a suspected genetic disease	23	30%	22%	22%	12
2018	90	Cohort	rWES	Acutely ill children with a suspected genetic disease	40	53%	30%	8%	16
	25	Cohort	rWGS	Infants with a suspected genetic disease	42	43%	31%	26%	23
	63	Cohort	rWGS	Children in a PICU or cardiovascular ICU	24	42%	13%	NE	9
	77	RCT	rWGS, SOC	Infants <4 months old with a suspected genetic disease	32	41%	31%	NE	13
2019	24	Cohort	rWES	Infants in a NICU with a suspected genetic disease	25	72%	60%	NE	7.2
	111	Cohort	rWES	Children in a PICU or other ICU with a suspected genetic disease	40	53%	43%	NE	6
	28	Cohort	rWGS	Children with a suspected genetic disease	195	21%	13%	NE	21
	81	Cohort	rWGS	Children aged 4 months to 18 years with a suspected genetic disease	38	48%	39%	8%	14
	16	Cases	urWGS	Infants in an ICU with a suspected genetic disease	7	43%	43%	NE	0.8
2020	12	Cohort	rWES	Children <6 years old in a PICU with a new metabolic or neurologic disease	10	50%	30%	NE	9.8
	15	Cohort	rWES	Inpatient children >1 year old in an ICU	102	31%	27%	NE	11
	27	Cohort	rWES	Children with a critical illness selected by medical genetics	46	43%	52%	NE	9
	31	Cohort	rWES	Infants <6 months old in an ICU with hypotonia, seizures, metabolic abnormalities, or multiple congenital anomalies	50	58%	48%	NE	5
	79	Cohort	rWES	Various	41	32%	NE	NE	7
	86	Cohort	rWES	Infants in a NICU or PICU with a suspected genetic disease	18	83%	61%	NE	14
	87	Cohort	rWES	Infants in an ICU	368	27%	NE	NE	NE
	98	Cohort	rWES	Children and infants in a NICU or PICU with complex symptoms	130	48%	23%	NE	3.8
	99	Cohort	rWES	Infants in a NICU or PICU with a suspected genetic disease	33	70%	30%	30%	1
	2	Implementation	rWES	Children <18 years old in a NICU or PICU	108	51%	44%	NE	3
2019–2020	11, 23, 43	RCT	rWES	Infants with a disease of unknown etiology within 96 h of admission	95	20%	20%	18%	11
			rWGS		94	19%	24%	10%	11
			urWGS		24	46%	63%	25%	4.6

(*Continued*)

Table 2 (*Continued*)

Year(s)	Reference(s)	Study type	Test type(s)	Enrollment criteria	Size	Diagnostic rate	Change in management	Change in outcome	TAT (days)
2021	73	Cohort	rWES	Critically ill infants and children aged 6 days to 15 years with a suspected genetic disease	40	43%	31%	NE	5
	84	Cohort	rWES	Infants in a NICU or PICU with a suspected genetic disease	61	43%	11%	NE	60
	21	Cohort	rWGS	Infants in a NICU or PICU with a probable genetic disease and in urgent need of etiologic diagnosis to guide medical care	37	57%	NE	NE	43
	110	Crossover	rWES	Critically ill infants with conditions suggestive of a genetically heterogeneous disorder	202	20%	NE	NE	20
			rWGS		202	37%	7%	NE	7
	58	Crossover	rWGS, panel	Infants with a disease of unknown etiology	113	33%	26%	NE	NE
	22	Implementation	rWGS	Medicaid-covered infants with a disease of unknown etiology within 1 week of admission	178	43%	31%	NE	3
	72	RTDCT	rWGS, WGS	Infants 0–120 days old in an ICU with a suspected genetic disease	354	31%	25%	NE	15
Total/weighted average					2,874	36%	27%	18%	NE

Abbreviations: ICU, intensive care unit; NE, not evaluated; NICU, neonatal intensive care unit; PICU, pediatric intensive care unit; RCT, randomized controlled trial; RTDCT, randomized time-delayed clinical trial; rWES, rapid whole-exome sequencing; rWGS, rapid whole-genome sequencing; SOC, standard of care; TAT, turnaround time; urWGS, ultrarapid whole-genome sequencing; WGS, whole-genome sequencing.

(range 19–83%, $n = 2,874$). Twenty-nine studies ($n = 2,222$) also evaluated acute clinical utility, as measured by changes in management upon return of results. The weighted average rate of change in management was 27% (range 7–63%). Ten studies ($n = 487$) examined changes in outcome following those changes in management. The weighted average rate of change in outcome was 18% (range 8–30%).

A meta-analysis compared the diagnostic and clinical utility of WGS, WES, and chromosomal microarray analysis in 20,068 children with suspected genetic diseases through August 2017 (17). It found that the diagnostic utility of WGS [41%, 95% confidence interval (CI) 34–48%] was not significantly different from that of WES (36%, 95% CI 33–40%) but was greater than that of chromosomal microarray analysis (10%, 95% CI 8–12%). Diagnosis was significantly more likely for trios than for singletons (odds ratio 2.04, 95% CI 1.62–2.56). Interestingly, the rate of diagnosis by WGS or WES was higher for hospital-based interpretation (42%, 95% CI 38–45%) than for interpretation by reference laboratories (29%, 95% CI 27–31%). WGS had greater clinical utility (27%, 95% CI 17–40%) than chromosomal microarray analysis (6%, 95% CI 5–7%).

RCT: randomized controlled trial

NSIGHT: Newborn Sequencing in Genomic Medicine and Public Health

PICU: pediatric intensive care unit

There have been three randomized controlled trials (RCTs) of rWGS in infants in ICUs. The first, Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT), compared the rate of genetic disease diagnosis with rWGS plus standard genetic tests to that of standard genetic tests alone (including exome sequencing) in 65 infants aged <4 months in a regional NICU or pediatric intensive care unit (PICU) with illnesses of unknown etiology (77). The study was terminated early due to loss of equipoise: 15% of controls underwent compassionate crossover to receive rWGS, demonstrating the difficulty of diagnostic RCTs. The rate of genetic diagnosis within 28 days of enrollment (the primary end point) was higher with rWGS (31%) than with controls (3%). Median time to diagnosis was significantly less with rWGS (13 days) than with

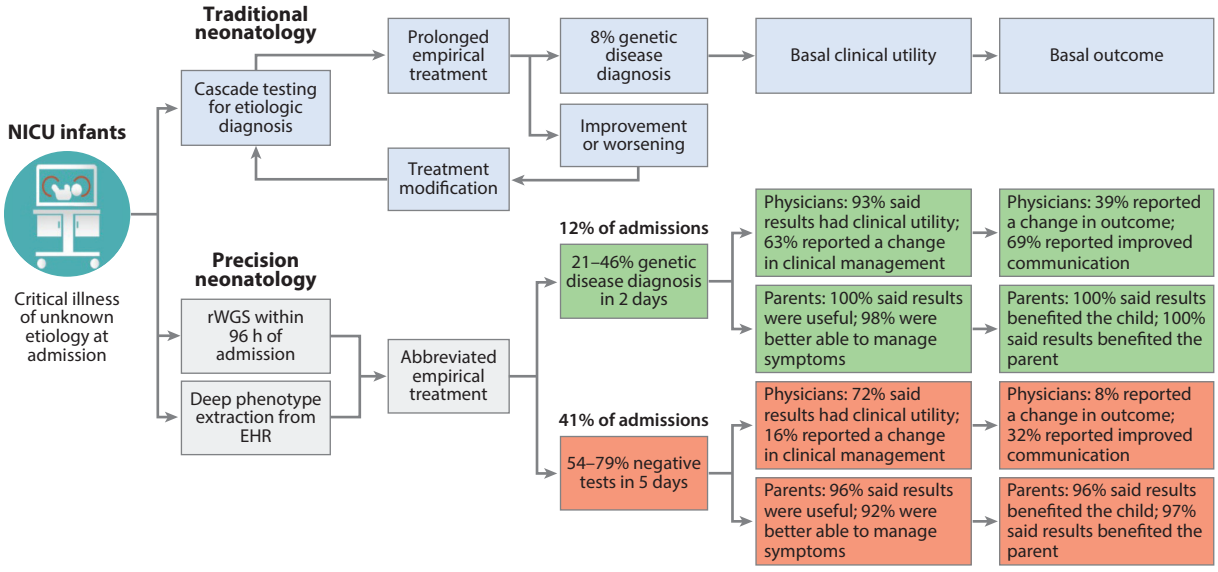

Figure 3

Comparison of the traditional approach to etiologic diagnosis of genetic diseases in NICU infants with rWGS-informed precision medicine. Values are from the NSIGHT2 RCT (11, 23, 43). Abbreviations: EHR, electronic health record; NICU, neonatal intensive care unit; NSIGHT, Newborn Sequencing in Genomic Medicine and Public Health; RCT, randomized controlled trial; rWGS, rapid whole-genome sequencing. Figure adapted with permission from Reference 23.

controls (107 days). This study established that rWGS increased the proportion of NICU/PICU infants who received timely diagnoses of genetic diseases relative to standard genetic tests.

The second RCT, NSIGHT2, evaluated the effectiveness of rWES, rWGS, and urWGS as first-tier tests in 213 seriously ill infants with diseases of unknown etiology, representing 46% of NICU admissions (a broader proportion than previously studied) (43). The analytic performance of rWGS/urWGS was superior to that of rWES (**Table 1**). The rWES and rWGS diagnostic rates and times to result were the same (~20% and median of 11 days, respectively). Both, however, were inferior to those of urWGS (diagnostic rate 46%, median time to result 4.6 days). The incremental diagnostic yield of reflexing to trio after negative singleton analysis was only 0.7%, a result that was at odds with the previously published meta-analysis. A second report from the NSIGHT2 RCT demonstrated that clinicians perceived rWGS to be useful (the primary NSIGHT2 study end point) in 77% of infants (23) (**Figure 3**). Interestingly, both positive (93%) and negative (72%) tests had clinical utility. The explanation of the latter was that negative rWGS results were useful in decreasing the posterior probability of genetic disease, enabling clinicians to focus on nongenetic etiologies. The results of rWGS changed clinical management in 28% of infants and outcomes in 15%.

A third report from the NSIGHT2 RCT evaluated parental perceptions of clinical utility, adequacy of consent, and potential harms and benefits (11) (**Figure 3**). When rWGS was first introduced, there were concerns that parents of newborns in ICUs would be unable to provide informed consent and that testing would cause anxiety, lead to decisional regret or depression, and interfere with bonding. However, more than 90% of NICU infant parents felt adequately informed to consent to diagnostic genome sequencing. Although only 23% of infants received a diagnosis, 97% of parents reported that genome sequencing was useful, and the median decisional regret was 0 (on a scale of 0–100). Evaluation of potential harms of testing revealed that only 2%

perceived harm (1% related to a negative result and 2% related to stress or confusion). This study established that when rWGS was performed as a first-tier diagnostic test in almost half of regional NICU infants, most results were considered useful, and harms were rare and mild.

The third RCT was a multicenter, time-delayed trial of 354 infants aged <4 months in ICUs who had a suspected genetic disease and were randomized to receive WGS results within either 15 or 60 days after enrollment (72). Among infants who received WGS results within 15 days, 31% had diagnoses, and 21% had consequent changes in management at 60 days after enrollment, compared with 15% and 10%, respectively, among those who received results within 60 days. This study established the superiority of using rWGS as a first-tier diagnostic test.

In summary, research studies have established an evidence base for the diagnostic and clinical utility of rWGS as a first-tier diagnostic test for NICU infants with diseases of unknown etiology. This evidence base contributed to the publication by the American College of Medical Genetics and Genomics of a clinical guideline that supports the clinical utility and desirable effects of WES and WGS in active and long-term clinical management for pediatric patients less than 1 year of age with 1 or more congenital anomalies (56).

IMPLEMENTATION SCIENCE AND QUALITY IMPROVEMENT

While research studies are ideal for testing hypotheses, they feature biases that can lead to their results' failing to be repeated in general experience. Inclusion and exclusion criteria and the requirement for informed parental consent, for example, can result in enrollment that is not representative of NICU populations. Furthermore, research studies employ staff such as genome-literate research nurses and genetic counselors who may upskill NICU teams, enabling practices that would not be possible in their absence.

The translation of novel, evidence-based practices into routine clinical use typically takes decades, but the field of implementation science has developed strategies to facilitate adoption of evidence-based practices (60, 78, 102, 103). Implementation science—"the scientific study of methods to promote the systematic uptake of research findings and other EBPs [evidence-based practices] into routine practice, and, hence, to improve the quality and effectiveness of health services" (4, p. 1)—involves comprehensive identification of the key barriers to adoption, the organization of these barriers within a framework, and then real-world implementation studies that explore how to refine implementation to optimize effectiveness and facilitate adoption. Sites in the United States, the United Kingdom, and Australia started implementation science studies of rWGS in 2017, motivated by the desire to scale use by NICU teams and achieve sustainable improvement in outcomes (13).

The resultant framework developed by Rady Children's Institute for Genomic Medicine is shown in **Figure 4** (45). Assessment of barriers to adoption across approximately 80 NICUs served by the institute revealed the need to implement rWGS within a learning system for delivering rapid precision medicine. Based on the successful framework for implementation of traditional newborn screening (7), this system had four components: education, engagement, and equipping (items 1–6 in **Figure 4**, which include the upskilling of NICU teams, onboarding of health systems, and early identification of infants in need during admission); diagnostic rWGS (items 7–14, which include rWGS ordering, sequencing, interpretation, results review with ordering pediatricians, and quality improvement); translation into precision neonatology (items 15 and 16, comprising acute management guidance and precision medicine delivery); and therapeutic innovation (items 17–20, which include outcome monitoring, data analytics, natural history studies, and real-world evidence gathering) (45).

Implementation science studies of rWGS have started to be published. For example, Project Baby Bear, a payer-funded implementation project, evaluated the clinical and economic impact

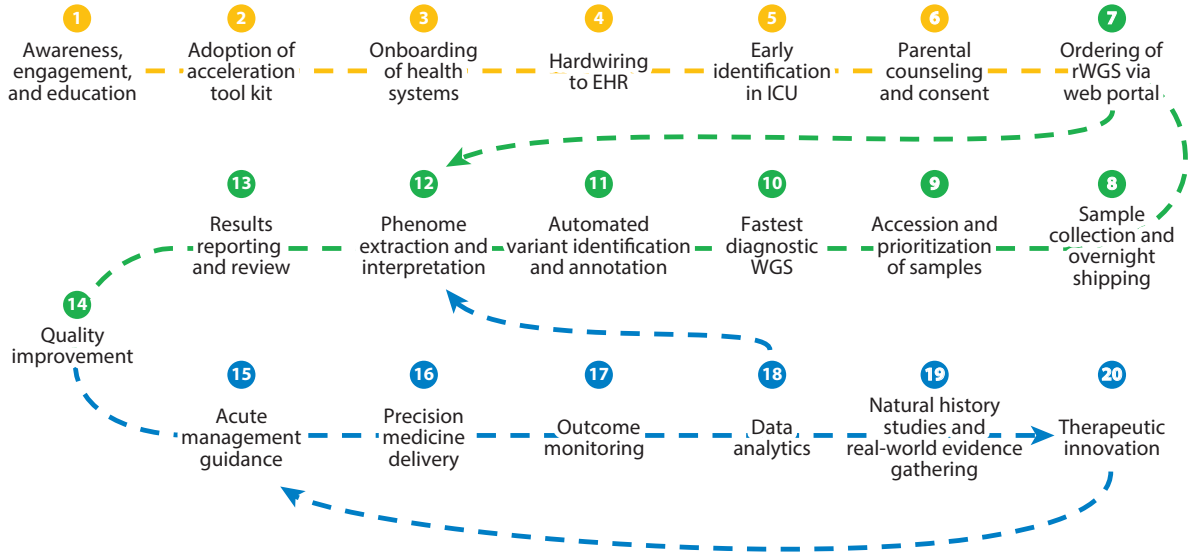

Figure 4

Genome-informed neonatology as part of a learning healthcare delivery system with four components: education, engagement, and equipping (items 1–6; *yellow line*); diagnostic rWGS (items 7–14; *green line*); translation into precision neonatology (items 15 and 16; *blue line*); and therapeutic innovation (items 17–20; *blue line*). The reverse blue arrows from item 18 to item 12 and from item 20 to item 15 indicate learning feedback loops. The green arrow from item 7 to item 12 indicates EHR-derived phenotype data that are integrated with genotype data at time of interpretation. Abbreviations: EHR, electronic health record; ICU, intensive care unit; rWGS, rapid whole-genome sequencing; WGS, whole-genome sequencing.

of the Rady Children's Institute for Genomic Medicine system of rWGS-based rapid precision medicine (22, 26). rWGS was utilized as a first-line diagnostic test in Medicaid-covered infants with diseases of unknown etiology in five regional California NICUs. The majority of infants were from underserved populations. Of 184 infants enrolled, 40% received a diagnosis by rWGS that explained their admission, with a median turnaround time of 3 days. In 32% of infants, rWGS led to changes in medical care. rWGS testing and the resultant precision medicine cost $1.7 million (~$21,000 per diagnosis) but led to ~$2.5 million in cost savings ($13,526 per infant tested; **Figure 5*a***). Sensitivity analysis showed that most of the cost savings were lost if the turnaround time was lengthened to 14 days (**Figure 5*a***). Inclusive of savings for families and ongoing cost of care, cost savings were ~$3.7 million ($19,935 per infant tested; **Figure 5*b***). Thus, Project Baby Bear confirmed the diagnostic and clinical utility previously shown in research studies and demonstrated a net reduction in healthcare utilization costs associated with relatively broad indications for first-tier rWGS. Analysis of the implementation process revealed (*a*) the need for an rWGS champion in each NICU, (*b*) educational needs and strategies, (*c*) the need to negotiate decision-making roles and processes, (*d*) workflows and workarounds, and (*e*) perceptions about rWGS.

A similar implementation pilot in 12 hospitals demonstrated the feasibility of rWES with a 3-day turnaround time in critically ill pediatric patients with suspected monogenic conditions in the Australian public health care system (2). Genetic diseases were diagnosed in 51% of infants, and management was changed in 76% of those receiving diagnoses and 11% of infants with negative reports. An earlier implementation project by the same group in two Australian centers showed similar rates of diagnostic and clinical utility, with a cost per diagnosis of $10,453 and a net cost savings of $10,600 per infant tested (90).

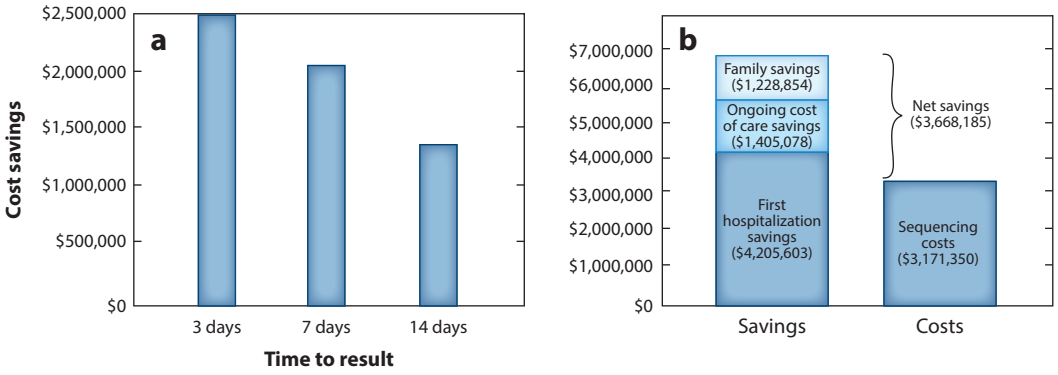

Figure 5

Relationship between the cost-effectiveness of precision neonatology and time to result. (*a*) Cost savings during the initial hospitalization from first-tier use of rWGS for NICU infants with suspected genetic diseases in Project Baby Bear. (*b*) Total cost savings. Abbreviations: NICU, neonatal intensive care unit; rWGS, rapid whole-genome sequencing. Data are from Reference 22.

POLICIES AND GUIDELINES

Three national health systems (the National Health Service in England and in Wales and Australian Genomics) are adopting rWGS in critically ill inpatient infants with suspected genetic diseases. In July 2019, Blue Shield of California became the first major payer to issue a coverage policy for diagnostic rWES and rWGS in NICU infants and trios (9) (see the sidebar titled Blue Shield of California Policy for Coverage of Rapid Whole-Exome and Whole-Genome Sequencing in Infants in Intensive Care Units). Rapid testing was defined as an average turnaround time of less than 14 days, but usually less than 7 days. The policy called for immediate verbal reporting of results to the clinician if changes in management were likely. The criteria for reimbursable

BLUE SHIELD OF CALIFORNIA POLICY FOR COVERAGE OF RAPID WHOLE-EXOME AND WHOLE-GENOME SEQUENCING IN INFANTS IN INTENSIVE CARE UNITS

The July 2019 Blue Shield of California policy for the use of rWES and rWGS for infants in ICUs read as follows (9):

> Rapid whole exome sequencing or rapid whole genome sequencing, with trio testing when possible, **meets the definition of medical necessity** for the evaluation of critically ill infants in neonatal or pediatric intensive care with a suspected genetic disorder of unknown etiology when **both (1 & 2)** of the following criteria are met:
>
> 1. **At least one** of the following criteria is met:
>   a. Multiple congenital anomalies (e.g., persistent seizures, abnormal ECG, hypotonia);
>   b. An abnormal laboratory test or clinical features suggests a genetic disease or complex metabolic phenotype (e.g., abnormal newborn screen, hyperammonemia, lactic acidosis not due to poor perfusion); **or**
>   c. An abnormal response to standard therapy for a major underlying condition.
> 2. **None** of the following criteria apply regarding the reason for admission to intensive care:
>   a. An infection with normal response to therapy;
>   b. Isolated prematurity;
>   c. Isolated unconjugated hyperbilirubinemia;
>   d. Hypoxic Ischemic Encephalopathy;
>   e. Confirmed genetic diagnosis explains illness;
>   f. Isolated Transient Neonatal Tachypnea;
>   g. Nonviable neonates.

testing (given in the sidebar) reflect those used in published research studies such as Project Baby Bear and NSIGHT2. A new Current Procedural Terminology (CPT) code, 0094U, was authorized for rWGS. In March 2020, the policy was accepted by Anthem Blue Cross Blue Shield and has since been ratified by 10 regional plans. In September 2021, Michigan became the first US state to reimburse for rWGS in critically ill Medicaid-covered infants in NICUs and PICUs as a carve-out from the intensive care diagnosis-related group (DRG) codes. The Michigan coverage policy is very similar to that of Anthem Blue Cross Blue Shield. The CPT code 0094U was implemented and priced at $6,275 for probands and $10,750 for trios. In July 2021, Governor Newsom signed California Assembly Bill 114 into law, providing $6 million to reimburse rWGS in Medicaid-covered infants in California in 2022. A coverage policy has not yet been issued by the California Department of Health. Feasibility projects of rWES and rWGS are underway in NICU infants in many high- and middle-income countries (89).

CPT: Current Procedural Terminology

DRG: diagnosis-related group

FUTURE DIRECTIONS

Integration of Omics Technologies, Increased Use of Whole-Exome and Whole-Genome Sequencing, and Development of Precision Therapies

Many research and implementation studies are currently underway that will continue to refine our understanding of the use of WGS in NICUs and other pediatric intensive care settings (PICUs and pediatric cardiac ICUs). It is already apparent, however, that rWGS-based precision medicine has considerable diagnostic and clinical utility and cost-effectiveness in infants in ICUs. This evidence will drive an era of broad adoption of rWGS-informed precision medicine for infants in NICUs. We anticipate the issuance of guidelines from professional bodies that support use of rWGS as a first-tier diagnostic test in critically ill infants with diseases of unknown etiology. We further anticipate that the guidelines will recommend testing shortly after admission, a turnaround time of 3 days, and reports that include all classes of variants, and that rWGS-informed precision medicine will become part of the core curriculum for neonatology fellowship. In parallel, there will continue to be broader reimbursement of rWGS by Medicaid and private payers. It will be important for coverage policies to include reimbursement as a carve-out payment rather than in current DRGs.

A key unanswered question is how broadly WGS should be used in NICUs. The NSIGHT2 study showed diagnostic and clinical utility when WGS was used in 46% of admissions to a regional NICU. This was the first study to quantify the value of negative WGS. Further studies are needed to examine optimal breadth of use in level II, III, and IV NICUs.

The research published to date still underestimates the prevalence of single-locus genetic diseases in infants in ICUs. An unpublished study of postmortem infants from one of us (S.F.K.) found a high rate of undiagnosed genetic disorders (M.J. Owen, M.S. Wright, S. Batalov, Y. Kwon, Y. Ding, et al., manuscript in review), many of which had effective treatments in a single US county with relatively broad use of rWGS in NICUs and PICUs (45, 82). Genetic diseases continue to be discovered at a rapid pace, and the quality of WGS continues to improve rapidly. For example, it is likely that diagnostic yield will increase by 5–15% through use of combined long- and short-read WGS (64). Long-read WGS is more expensive than short-read WGS, and although it is not as good at detecting single-nucleotide variants, it is far superior for characterizing structural variants and copy number variants. The emerging picture of these variants is that they are generally more complex than we were aware of and frequently are combination events that include deletions, insertions, and rearrangements at a single locus.

In addition, in the relatively near future, we will change from read alignment to read assembly, generating assembled individual genomes (85). It is likely that this will increase yield by another 5–15%, particularly for individuals from racial and ethnic groups that were not included in the

AI: artificial intelligence

FDA: Food and Drug Administration

current reference human genome. An intermediary to genome assemblies may be digital, automated ethnic ancestry delineation in short-read WGS and then alignment to the best from a large set of diverse reference genomes (37).

Lastly, as we enter a new era of integrative clinical omics, we are starting to see the value of integrating WGS with functional omics, such as RNA sequencing (51), proteomics, and metabolomics. Currently, there are a large number of variants for which we cannot predict pathogenicity, most of which are intergenic or intronic. These omics technologies allow us to evaluate the functional consequences of such variants. The two uses of such technology are to diagnose unsolved cases and to build new databases of functional omics-annotated variants. Ultimately, there will be very few remaining VUSs.

Another trend that is gaining momentum is the use of artificial intelligence (AI) to improve scalability and increase adoption of rWGS and precision medicine in infants in ICUs. In addition to the emerging uses of AI discussed previously, we anticipate algorithm-based, automated electronic health record (EHR) alerts triggered in infants with a high likelihood of underlying genetic disease and a high risk of mortality (akin to EHR sepsis alerts) (80). A recent study described the development of an AI-informed, automated system for provision of acute management guidance for newly diagnosed genetic diseases (74). This system was designed for use by front-line intensivists and neonatologists, particularly in hospitals that lack a full array of subspecialists and superspecialists, where there may be delays in implementation of optimal treatments for ultrarare genetic diseases. AI tools such as these will be critical in bringing rWGS-based precision medicine to most birthing-hospital-associated NICUs.

The most exciting future advance will be accelerated development of gene therapies (**Figure 1**). Effective gene therapy for spinal muscular atrophy type 1 is a staggering accomplishment. We are realizing, however, that the system shown in **Figure 4** is critically needed in order to diagnose and treat hypotonic babies in the first week of life. A by-product of increasing use of rWGS is unparalleled natural history studies of specific infant-onset genetic diseases that accelerate drug development both by identifying end points and indications and by serving as real-world evidence for submissions of investigational new drug applications to the US Food and Drug Administration (FDA) (95). It must be understood that the majority of infant-onset genetic diseases either were only recently discovered or were not generally diagnosable in the absence of rWGS. Thus, many genetic diseases for which drug development was not previously feasible now have potential therapeutic strategies, including gene therapy, antisense oligonucleotides, small molecules, genome editing, and repurposed FDA-approved drugs. In addition to better therapies, natural history studies will improve our ability to predict the prognosis for critically ill infants, communicate with parents, anticipate complications, and stratify patients within genetic disorders, as is now happening with Duchenne muscular dystrophy and cystic fibrosis.

A final area, but one that is beyond the scope of this review, is newborn screening for genetic diseases by rWGS (8) and increasing use of rWGS for fetal diagnosis. We anticipate that, within the next five years, healthy newborns will start to be widely screened by WGS for approximately 500 severe infant-onset genetic diseases that have effective treatments. Professional society opinions support the use of prenatal WES in cases with specific fetal anomalies, and we anticipate imminent testing of fetal rWGS to improve diagnostic success (18, 34, 68, 94, 100).

Next Steps: Broad Implementation of Genome Sequencing and Associated Research

Genetic heterogeneity of fetal and neonatal phenotypes, overrepresentation of novel and rare genomic variants in the NICU population, the high fraction of affected infants in NICUs with undiagnosed congenital anomalies or metabolic disorders, the significant contribution of genetic

disorders to morbidity and mortality among infants, and the proven record of rWES and rWGS to change clinical management and identify individualized therapeutic strategies make the integration of rWGS into NICU clinical care a high priority (102, 103). As illustrated by the framework developed by Rady Children's Institute for Genomic Medicine described above (45), acceleration of this integration will require an intentional commitment to implementation of a sustainable genomic learning healthcare system through implementation science that uses knowledge and experience derived from NICU and prenatal clinical care for cycles of continuous improvement and research to improve patient outcomes through enhanced diagnostic success, clinical efficiency, and discovery of genotype-guided therapeutics (36, 60, 61, 102, 103, 109). Multiple stakeholders—including parents, payers, bioinformaticians, geneticists, genetic counselors, neonatologists, obstetricians, pediatric subspecialists, developmental biologists, model organism investigators, experts in strategies to rescue variant-encoded disruption (e.g., with gene therapy, antisense oligonucleotides, small molecules, genome editing, or repurposed FDA-approved drugs), clinical investigators, genomicists, and institutional leaders—will need to participate in the development of a genomic learning healthcare system for NICUs. The challenges in implementing this system include facilitating a cultural shift among providers from phenotype-first to genotype-first diagnosis, creating governance structures that can adapt to new ethical and operational questions, standardizing genomic and phenotypic information in EHRs, enabling more reliable computational and functional evaluation of novel and rare VUSs that are clinically actionable, and developing consent strategies that permit the inclusion of an individual's clinical and genomic data while protecting patient confidentiality (61, 101, 109). However, NICUs have a long record of commitment to quality improvement through implementation science and, more recently, of supporting value-based quality initiatives (32). With the already available evidence of the clinical value of r/urWES and r/urWGS in the NICU, implementation of a genomic learning healthcare system will lead to deployment of these best practices to improve outcomes for critically ill newborn infants and children and reduce the costs of their care.

DISCLOSURE STATEMENT

S.F.K. is employed by Rady Children's Institute for Genomic Medicine, whose mission is to prevent, diagnose, treat, and cure childhood diseases through genomic and systems medicine research; he has also filed patents related to diagnostic rWGS.

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