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ARTICLE

An RCT of Rapid Genomic Sequencing among Seriously Ill Infants Results in High Clinical Utility, Changes in Management, and Low Perceived Harm

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Summary

The second Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT2) study was a randomized, controlled trial of rapid whole-genome sequencing (rWGS) or rapid whole-exome sequencing (rWES) in infants with diseases of unknown etiology in intensive care units (ICUs). Gravely ill infants were not randomized and received ultra-rapid whole-genome sequencing (urWGS). Herein we report results of clinician surveys of the clinical utility of rapid genomic sequencing (RGS). The primary end-point—clinician perception that RGS was useful— was met for 154 (77%) of 201 infants. Both positive and negative tests were rated as having clinical utility (42 of 45 [93%] and 112 of 156 [72%], respectively). Physicians reported that RGS changed clinical management in 57 (28%) infants, particularly in those receiving urWGS ($p = 0.0001$) and positive tests ($p < 0.00001$). Outcomes of 32 (15%) infants were perceived to be changed by RGS. Positive tests changed outcomes more frequently than negative tests ($p < 0.00001$). In logistic regression models, the likelihood that RGS was perceived as useful increased 6.7-fold when associated with changes in management (95% CI 1.8–43.3). Changes in management were 10.1-fold more likely when results were positive (95% CI 4.7–22.4) and turnaround time was shorter (odds ratio 0.92, 95% CI 0.85–0.99). RGS seldom led to clinician-perceived confusion or distress among families (6 of 207 [3%]). In summary, clinicians perceived high clinical utility and low likelihood of harm with first-tier RGS of infants in ICUs with diseases of unknown etiology. RGS was perceived as beneficial irrespective of whether results were positive or negative.

Introduction

Of the four million infants born in the US each year, 7%–10% are admitted to a neonatal intensive care unit (NICU), pediatric intensive care unit (PICU), or cardiovascular intensive care unit (CVICU) for diagnosis and treatment of an acute illness, with an increasing proportion being term and normal birthweight.[1–4] Although the majority of NICU admissions are associated with prematurity, hypoglycemia, and suspected infections, single-locus genetic diseases are common among infants in ICU settings.[1–7] Genetic disorders and malformations are the most common cause of infant mortality, accounting for more than a third of all deaths at one children's hospital.[8–10] Previous work has demonstrated that rapid genome-wide sequencing (ultra-rapid whole-genome sequencing [urWGS], rapid whole-genome sequencing [rWGS], or rapid whole-exome sequencing [rWES]) is associated with both a shorter time to diagnosis and an increased diagnostic yield when compared with standard-of-care testing, including gene panels and chromosome microarrays.[6,7,10–25] However, earlier diagnosis of genetic disorders is a process measure and not a definitive mark of clinical utility.

It is important when considering deployment of novel interventions in medicine to establish quantitatively their benefits and risks. Therefore, prior to broader uptake of genome-wide sequencing as a first line diagnostic test, effects on patient care should be carefully evaluated to establish in which situations, if any, benefits (such as improved patient and family outcomes) are outweighed by potential risks (such as high cost and/or psychosocial harm). However, the concept of benefit in medicine, or clinical utility, has been defined in a plurality of ways, stratified by multiple levels of benefit, corresponding to the perspectives of various stakeholders: children, parents, healthcare providers, laboratorians, hospital administrators, payers, and policymakers.[26–31] As such, generating and evaluating evidence of clinical utility is complex.[32] This challenge is compounded by the variety of clinical presentations in the NICU, PICU, and CVICU, the multitude of disorders that can be diagnosed with genome-wide sequencing, and a range of outcomes. To date, very little data have been gathered on the potential risks of the deployment of rapid genome-wide sequencing in infants in NICU, PICU, and CVICU settings.[26]

Although 15 studies to date have shown, by varying definitions, the clinical utility of rapid genome-wide

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sequencing in the NICU, PICU, and CVICU, it is not yet clear what the specific indications for such testing should be, nor, beyond case series, in what situations urWGS, rWGS, or rWES are associated with improved outcomes.[6,7,11–26,33] Moreover, modeling of previously published data suggests that both the cost and clinical utility of urWGS, rWGS, and rWES vary inversely with time to result.[14,16] However, the optimal match of presentations, timeliness, and cost effectiveness is not yet known. Finally, it not clear whether patient and family perceptions of improved outcomes match those of physicians. A focus group of neonatologists identified four themes regarding rWGS: (1) questions about the interpretation of results, (2) uncertainty about parental consent and limits on parental right to know genomic information, (3) different opinions about whether and how genomic results could be clinically useful, and (4) potential risks of diagnostic genomic sequencing.[34]

We undertook a prospective, randomized, controlled study to examine clinical and family-centered outcomes in acutely ill infants receiving urWGS, rWGS, or rWES as a first-tier test (NSIGHT2; ClinicalTrials.gov: NCT03211039).[6,35] Previously, we reported results from NSIGHT2 related to the analytic and diagnostic performance of singleton and trio rWES and rWGS.[6] The accompanying article[36] reports parental perceptions of benefits and harms. However, the primary hypothesis tested in NSIGHT2 was that rWGS has greater clinical utility for a subset of ill infants and their families than rWES. Herein, we report results of evaluation of three key outcomes. First, did the physician perceive utility in rapid genome-wide sequencing? Second, did the physician believe that the test led to a change in care? Third, was that change in care expected to improve short- and long-term outcomes? Further, we sought to identify physician concerns about the safety or potential harms of testing.

Subjects and Methods

Subjects and Study Design

NSIGHT2 was a prospective, randomized, controlled, blinded trial in infants in the NICU, PICU, and CVICU at Rady Children's Hospital, San Diego (RCHSD) which compared the clinical utility and outcomes of rWGS and rWES, with analysis as singleton probands and reflex to familial trios between 6/29/2017 and 10/9/2018 (ClinicalTrials.gov: NCT03211039, Figure S1).[6] NSIGHT2 was approved by the local institutional review board, was designated non-significant risk by the Food and Drug Administration in an Investigational Device Exemption presubmission, and was performed in accordance with the Declaration of Helsinki. Eligible patients in ICUs were identified by daily census review, while infants on the general pediatric units had to have eligibility review requested by their inpatient team. Informed consent was obtained from at least one parent or guardian. The inclusion criteria were age <4 months and <96 h elapsed since admission or development of a new feature that changed the differential diagnosis to include a genetic condition.[6] New features included abnormal responses to standard therapy for an underlying condition, development of new clinical features, or abnormal laboratory tests. Specifically excluded were infants with a previously confirmed genetic diagnosis and infants in whom the clinical presentation was accounted for by a common acquired etiology (isolated prematurity, transient tachypnea of the newborn, isolated unconjugated hyperbilirubinemia, sepsis with a normal response to therapy, or hypoxic ischemic encephalopathy with a clear precipitating event).[6] Infants who were gravely ill with presentations that included differential diagnoses with a potential change in management were excluded from randomization. They received urWGS, with the fastest possible time to diagnosis. All other infants were randomized to receive either rWES or rWGS.

Rapid Whole-Genome and -Exome Sequencing, Analysis, and Interpretation

Clinical urWGS, rWGS, and rWES methods used in NSIGHT2 have been published in detail.[6] In brief, experts selected clinical features representative of each child's illness from the Electronic Health Record. Trio EDTA-blood samples were obtained where possible. Genomic DNA was isolated with standard methods, fragmented by sonication, and bar-coded. Paired-end, PCR-free libraries were prepared for rWGS and urWGS with TruSeq DNA LT kits (Illumina) or Hyper kits (KAPA Biosystems). 2 × 101 nt rWGS and urWGS was performed to at least 40-fold coverage with Illumina instruments. Sample preparation and sequencing for rWES was performed by GeneDx. Targets were enriched with the xGen Exome Research Panel v1.0 (Integrated DNA Technologies).[6] FASTQ files for rWES were transferred to RCIGM for analysis and interpretation.

Sequences from urWGS, rWGS, and rWES were aligned to human genome assembly GRCh37 (hg19), and variants were identified with the DRAGEN Platform (Illumina).[6] Structural variants were identified with Manta and CNVnator and filtered to retain those affecting coding regions of known disease genes and with allele frequencies < 2% in the RCIGM database.[6] Copy number variants were identified in rWES data with the eXome-Hidden Markov Model software and utilizing exomes from 150 unaffected individuals to remove false positive calls.[37] Nucleotide and structural variants were automatically annotated and ranked using Opal Clinical (Fabric Genomics) and manually interpreted iteratively by clinical molecular geneticists according to standard clinical guidelines.[6] Genomic sequence interpretation was performed as singleton probands. Infants undiagnosed as singletons were reanalyzed as familial trios.[6] If testing identified a provisional diagnosis for which a specific treatment was available to prevent morbidity or mortality, this was immediately conveyed to the clinical team, as described. All causative variants were confirmed by Sanger sequencing, multiplex ligation-dependent probe amplification, or chromosomal microarray, as appropriate. Secondary findings were not reported, but medically actionable incidental findings were reported if families consented to receiving this information.

Clinician Survey

At study outset, no measures of clinical utility of genetic or diagnostic genomic sequencing had been validated for use in infant ICU populations. We therefore reviewed published tools that evaluated diagnostic thinking, therapeutic decision making, patient outcomes, and provider and patient perceptions of clinical utility. We identified tools that had been applied in newborn screening, NICUs, or genetics outpatient clinics. From these, we identified proxy measures of clinical decision making and process changes

and combined these with the types of changes in management observed in previous studies of genomic sequencing in ICUs.[12–14,16,17,19,26–33] From these, we devised 35 questions to evaluate benefit and harm perceived by clinicians and clinical teams (Supplemental Data). Draft questions were reviewed by NICU providers and pediatric subspecialists to ensure consistency and clarity. The primary study endpoint was clinician-perceived acute clinical utility of diagnostic genomic sequencing. This was measured by a 5-point Likert scale (not useful at all, not very useful, neutral, useful, or very useful). The responses "useful" and "very useful" were considered positive (testing had acute clinical utility). The survey contained 34 secondary questions in four domains: perceived specific changes in acute patient management (17 questions), perceived changes in outcomes (3 questions), changes in communication within ICU teams and with families (4 questions), changes in subsequent test ordering (4 questions), and changes in other care (6 questions, encompassing counseling, further monitoring, or research studies). Responses to the questions were compiled into 7 secondary endpoints. For statistical analysis, a positive response to any question related to that endpoint was considered positive.

The survey respondents were the clinicians caring for the infants when genomic sequencing results were returned (neonatologist, intensivist, primary admitting physician, consultant, neonatal nurse practitioner). Surveys were administered within 1 week of the return of results either in-person by a study team member or via electronic mail. More than one survey could be completed for each infant and it was possible to complete only part of the survey. For 20 infants (approximately one third) in whom healthcare providers reported changes in management on the questionnaire, the EHR was reviewed for documentation of those changes to examine the validity of responses.

Statistical Analysis

For each endpoint, three univariate analyses were performed: comparison of rates of occurrence following rWES versus rWGS, comparison of rates of occurrence following urWGS versus rWES and rWGS combined, and comparison of rates of occurrence following positive versus negative tests. Rates of occurrence were compared between groups with Fisher's exact test. Multiple logistic regression was used to evaluate which variables were associated with changes in management and clinician perception of clinical utility. Test type, result type, and turnaround time were included as predictor variables for both outcomes. Change in management was also included as a predictor variable in the model for clinical utility. Odds ratios and 95% confidence intervals are reported. A significance cutoff of $\alpha = 0.05$ was used for all analyses. All analyses were conducted in R v.3.3.3.

Results

NSIGHT2 was a prospective, randomized, controlled, blinded trial to compare the effectiveness (rate of diagnosis, time to diagnosis, clinician perceived utility, family perceived utility, and cost) and outcomes of two methods of rapid genomic sequencing (rWGS or rWES) and two methods of interpretation (singleton probands and familial trios) in acutely ill infants (Figure S1).[6] The NSIGHT2 inclusion criteria were infants aged less than 4 months, with disorders of unknown etiology, and within 96 h from admission to an RCHSD ICU, or from development of a new feature suggestive of a genetic condition (Figure S1).[6] Here we report results of the primary end-point—comparisons of perceived clinical utility by healthcare providers—and secondary end-points related to changes in management.

Subjects and Diagnoses

Previously we reported the clinical characteristics and demographics of enrolled infants, reasons for exclusion and failure to enroll, and results of analytic and diagnostic performance.[6] These are briefly reviewed here to provide context for clinical utility results. Between 6/29/2017 and 10/9/2018, 213 infants were enrolled, representing 17% of 1,248 infants screened and 37% of 578 eligible infants (Table 1).[6] A total of 95 (45%) infants were randomized to rWES and 94 (44%) to rWGS. A total of 24 infants (11%) were considered gravely ill and were not randomized, receiving urWGS instead. The rWGS and rWES groups did not differ in sex, race, ethnicity, age, birth weight, location, or intensity of medical therapy.[6] Infants who received urWGS differed from those who were randomized by older age at symptom onset (median day of life 3.1 days versus 0.5 days, respectively; $p = 0.03$), higher proportion receiving antibiotics (88% versus 44%, respectively; $p = 0.01$), and higher 28-day mortality (21% versus 2%, respectively; $p = 0.01$).[6] Including incidental findings, genomic sequencing identified genetic diseases in 51 (24%) of the 213 enrolled infants. Neither the diagnostic rate of trio and singleton sequencing nor that of rWGS and rWES differed significantly.[6] For urWGS, however, the diagnostic rate (11 of 24, 46%) and median time to positive report (2.3 days) were superior to those of rWES/rWGS (20%, $p = 0.01$, and 11.6 days, $p < 0.0001$, respectively).[6]

Clinical Utility of rWES, rWGS, and urWGS

Clinicians provided perceptions of the acute clinical utility of diagnostic genomic sequencing for 201 of the 213 infants enrolled (94% response rate). In 154 (77%) infants, diagnostic genomic sequencing was perceived to be useful or very useful, the primary NSIGHT2 end point. This did not differ significantly between infants randomized to rWES or rWGS or infants receiving urWGS (Table 1). A striking observation was the large number of infants in whom clinicians perceived negative genomic sequencing results to have had clinical utility (Figure 1). Despite this, however, the perceived clinical utility of positive genomic sequencing tests (those that identified a molecular diagnosis or incidental finding) was higher than that of negative tests (42 of 45, 93% versus 112 of 156, 72%, respectively; $p = 0.0023$).

Changes in Management and Outcome

Clinicians completed responses to the secondary end points, examined as binary questions, for 207 of the 213 infants enrolled (97% response rate). Clinicians responded that diagnostic genomic sequencing changed clinical management in 57 (28%) infants. The most frequently

Table 1. Univariate Analysis of Clinician Perception of Clinical Utility of rWGS, rWES, and urWGS in NICU, PICU, and CVICU Infants

	rWES	rWGS	rWES versus rWGS p Value	urWGS	urWGS versus rWES+ rWGS P value	Positive Tests	Negative Tests	Pos versus Neg Tests p Value
Infants enrolled[a]	95	94	N/A	24	N/A	51	162	N/A
Test identified molecular diagnosis or incidental finding, n (%)[a]	20 (21%)	20 (21%)	1	11 (46%)	0.01	N/A	N/A	N/A
Time to first positive or negative report (days), median (range)[a]	11.2 (4–39)	11.0 (3–49)	0.65	4.6 (1–4)	<0.0001	N/A	N/A	N/A
Time to first positive report (days), median (range)[a]	11.4 (8–39)	11.8 (3–25)	0.69	2.3 (1–14)	0.0002	N/A	N/A	N/A
Clinician Perception								
Surveys completed, n (%)	90 (95%)	93 (99%)	N/D	24 (100%)	N/D	49 (96%)	158 (98%)	N/D
Test was useful or very useful, n (%)	66 (76%)	66 (73%)	0.73	22 (92%)	0.07	42 (93%)	112 (72%)	0.002
Test changed management, n (%)	19 (21%)	23 (25%)	0.6	15 (63%)	0.0001	31 (63%)	26 (16%)	<0.00001
Test changed an outcome, n (%)	17 (19%)	9 (10%)	0.09	6 (25%)	0.22	19 (39%)	13 (8%)	<0.00001
Test improved communication, n (%)	34 (38%)	34 (37%)	0.88	16 (67%)	0.008	34 (69%)	50 (32%)	<0.00001
Test increased stress or confusion, n (%)	3 (35)	1 (1%)	0.36	2 (8%)	0.14	4 (8%)	2 (1%)	0.14
Test let to other changes in management, n (%)	20 (22%)	21 (23%)	1	10 (42%)	0.047	40 (82%)	11 (7%)	<0.00001
Test led to another test being cancelled, n (%)	16 (19%)	20 (22%)	1	8 (32%)	0.18	13 (27%)	31 (20%)	0.32
Test led to another test being ordered, n (%)	11 (12%)	15 (16%)	0.53	5 (21%)	0.37	19 (39%)	9 (6%)	<0.00001

N/A, not applicable; N/D, not done. Rates of occurrence were compared between groups with Fisher's exact test. A significance cutoff of $\alpha = 0.05$ was used for all analyses. All analyses were conducted in R v.3.3.3.
[a]Results were previously published.[6]

reported changes in management were screening for potential comorbidities associated with the genetic disease diagnosis (14 infants), new subspecialty consulted (14 infants), changes in medications (13 infants), changes in surgical interventions (9 infants), changes in diet (4 infants), changes in imaging studies (2 infants), and changes in palliative care (2 infants) (Supplemental Data). The occurrence of change in clinical management did not differ significantly between infants randomized to rWES and rWGS (Table 1). However, urWGS was associated with a significantly higher rate of change in management (15 of 24, 63%) than rWES/rWGS (42 of 183, 23%; $p = 0.0001$). Positive genomic sequencing tests were also associated with a significantly higher occurrence of change in management (31 of 49, 63%) than negative tests (26 of 158, 16%; $p < .00001$).

To evaluate the accuracy of clinician perceptions, we manually reviewed 20 (35%) randomly selected infant EHRs of the 57 infants in whom clinicians perceived that diagnostic genomic sequencing changed clinical management. There were 25 perceived changes in management in the 20 infants, including the addition of new medications, new surgical interventions and new subspecialty consultations, and changes in diet. Nineteen (95%) of 20 EHR reviews were consistent with clinician responses. The one disparity was in infant 216, who had microcephaly and an atrioventricular canal defect and who was small for gestational age; diagnostic genomic sequencing led to a new subspecialty consultation. EHR review indicated that the infant was transferred to another facility prior to return of results. Given the diagnosis of susceptibility to intracerebral hemorrhage (MIM: 614519), it was plausible that subspecialty consultation did occur at the outside institution.

Thirty-two (15%) of 207 clinician responses disclosed that diagnostic genomic sequencing changed infant outcomes (by targeted treatments in 21 [10%] infants, avoidance of complications in 16 [8%], and institution of palliative care in 2 [1%] infants). Changes in outcome did not differ significantly between infants randomized to rWES and rWGS, nor between urWGS and rWES/rWGS (Table 1). Positive genome sequencing tests, however,

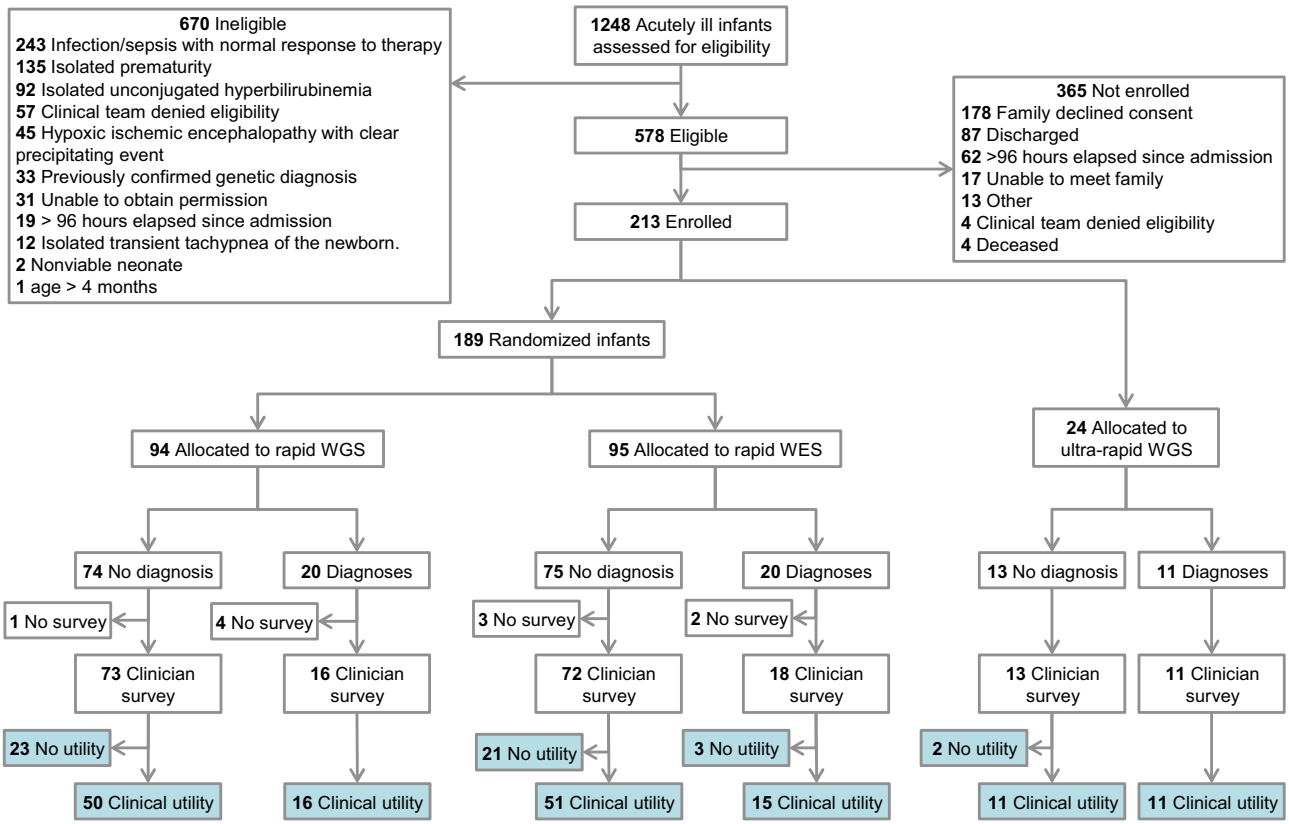

Figure 1. CONSORT Flow Diagram of Infants Who Were Screened for Eligibility in NSIGHT2, Sequenced, Received a Genetic Disease Diagnosis, and Were Surveyed for Clinician Perception of Clinical Utility
The primary end point was clinician perception that diagnostic genomic sequencing was useful or very useful (clinical utility or no clinical utility, blue shading).

were associated with significantly more changes in outcome (19 of 49, 39%) than negative tests (13 of 158, 8%; $p < 0.00001$).

Changes in Communication and Confirmatory Testing

At the outset of NSIGHT, there was considerable concern that genomic sequencing might be harmful in infants receiving intensive care.[35] Parental perceptions of benefits and harms in the NSIGHT2 study are reported in the accompanying article.[36] Here we report clinician perceptions of benefits and harms of genomic sequencing in infants. Of 207 clinician responses, 84 (41%) disclosed that diagnostic genomic sequencing improved communication with families regarding infant outcomes, expectations, and prognosis. Improved communication did not differ between infants randomized to rWES and rWGS (Table 1). Interestingly, communication was perceived to be improved in a greater proportion of infants receiving urWGS (16 of 24, 67%) than rWES/rWGS (68 of 183, 37%; $p = 0.008$). Improved communication was reported for those infants with positive genome sequencing tests were perceived to have (34 of 49, 69%) more often than those with negative tests (50 of 158, 32%; $p < 0.00001$). Three questions examined clinician-perceived increased stress or confusion among families or clinical staff as a result of genomic sequencing. Clinicians perceived increased stress or confusion in 6 (3%) of 207 cases. Increased stress did not differ significantly between rWES and rWGS, urWGS and rWGS/rWES, or positive and negative tests (Table 1).

At the outset of NSIGHT, there was also concern that genomic sequencing might lead to numerous confirmatory tests.[35] Eight questions examined the impact of genomic sequencing on additional testing, either for confirmation of a diagnosis or an incidental finding or a potential comorbidity. Of 207 clinicians, 44 (21%) reported that diagnostic genomic sequencing led to cancellation of other tests, and 31 (15%) reported addition of new tests. There were no differences between rWGS and rWES in subsequent tests cancelled or added (Table 1). urWGS did not lead to more test cancellations than rWGS/rWES nor more added tests (Table 1). Positive results did not lead to cancellation of more tests than negative results (13 of 49 [27%] versus 31 of 158 [20%], $p = 0.32$) but did lead to more added tests (19 of 49 [39%] versus 9 of 158 [6%], $p < 0.00001$).

Seven questions examined clinician perception of the impact of genomic sequencing on other changes in care (genetic and reproductive counseling, clinical monitoring or genetic testing of family members, and enrollment in

Table 2. Logistic Regression Analysis of Clinician Perception of Change in Management and Clinical Utility of rWGS, rWES, and urWGS in NICU, PICU, and CVICU Infants

Independent Variable	Description	Odds Ratio (95% CI)	p Value
Outcome: Change in Management			
Test type	rWES	reference	–
	rWGS	1.41 (0.65–3.1)	0.39
	urWGS	2.37 (0.72–7.75)	0.15
Result type	negative	reference	–
	positive	10.07 (4.74–22.35)	<0.0001
Turnaround time	days, accession to 1st report	0.92 (0.85–0.99)	0.03
Outcome: Clinical Utility			
Test type	rWES	reference	–
	rWGS	0.77 (0.38–1.57)	0.48
	urWGS	1.79 (0.41–12.61)	0.48
Result type	negative	reference	–
	positive	3.22 (0.98–14.71)	0.08
Change in management	no	reference	–
	yes	6.69 (1.83–43.3)	0.01
Turnaround time	days, accession to 1st report	0.98 (0.93–1.05)	0.59

CI, confidence intervals. Multiple logistic regression was used to evaluate which variables were associated with changes in management and clinician perception of clinical utility. Test type, result type, and turnaround time were included as predictor variables for both outcomes. Change in management was also included as a predictor variable in the model for clinical utility. Odds ratios and 95% confidence intervals are reported. A significance cutoff of $\alpha = 0.05$ was used for all analyses. All analyses were conducted in R v.3.3.3.

additional research studies). In 51 cases (25%), clinicians reported that diagnostic genomic sequencing changed one or more of these aspects of care. There were no differences between rWGS and rWES in other care changes. urWGS resulted in more changes in other care than rWES/rWGS (10 of 24 [42%] versus 41 of 183 [22%], respectively, $p = 0.047$). Positive results led to more changes in other care versus negative results (40 of 49 [82%] versus 11 of 158 [7%], respectively, $p < 0.00001$).

Predictors Associated with Clinician Perception

We used multiple logistic regression to evaluate which variables were associated with changes in management (Table 2). It should be noted that turnaround time (TAT) and test type were not completely independent. The median TAT of urWGS was 4.6 days (range 1.1–14 days), whereas it was 11.1 days with rWES and rWGS (range 3.3–49.1 days). Positive results were associated with change in management (OR 10.07, 95% CI 4.74–22.35), whereas TAT was inversely associated with change in management (OR 0.92, 95% CI 0.85–0.99) after accounting for test type.

We also used multiple logistic regression to evaluate which variables were associated with clinician perception that diagnostic genomic sequencing had clinical utility (Table 2). Change in management was significantly associated with clinical utility (OR 6.69, 95% CI 1.83–43.3) after accounting for result type (positive or negative), test type (rWES, rWGS, or urWGS), and TAT.

Discussion

The NSIGHT2 study was intended to explore optimal methods of genomic sequencing, and the scope and timing of the tests' use among infants in ICUs.[6] Enrollment was purposefully very broad: approximately 98% of 1,248 infants aged less than 4 months were screened at ICU admission.[6] 46% had illnesses of unknown etiology and, thus, were eligible for enrollment.[6] To ensure that genomic sequencing was a first-tier diagnostic test, eligibility was limited to the first 96 h of admission (or within 96 h of development of a new clinical feature that reset the diagnostic clock).[6] Another notable feature of NSIGHT2 compared to prior studies was the use of clinician surveys to generate detailed data of clinical utility, changes in management, and outcomes following genomic sequencing. The surveys had a 94% response rate for the primary end point and 97% response rate for the secondary end points, indicating representation of all study participants.

Ours is the first study to examine physician perception of clinical utility of genomic sequencing of infants in ICUs. The primary end point of NSIGHT2, and the dominant finding reported herein, was that clinicians perceived diagnostic genomic sequencing to be useful or very useful in 77% of infants tested. This proportion was higher than expected, especially in light of the relatively low diagnostic rate among randomized infants (21%). Importantly, clinicians did not perceive significant differences in the utility

Table 3. Prior Studies of the Diagnostic and Clinical Utility and Change in Outcome of rWES, rWGS, and urWGS in Children in ICUs

Reference	Date	Study Type	Sequencing Type	Neonatal and Pediatric Intensive Care Unit (NICU, PICU) Enrollment Criteria	Study Size	Rate of Diagnosis	Rate of Change in Management	Rate of Change in Outcome	Time to Result (days)
11	2012	cases	urWGS	NICU infants with suspected genetic disease	4	75%	N/D	N/D	2
12,13	2015	cohort	rWGS	<4 mo of age; suspected actionable genetic disease	35	57%	31%	29%	23
14	2017	cohort	rWES	<100 days of life; suspected genetic disease	63	51%	37%	19%	13
15	2018	RCT	rWGS	<4 mo of age; suspected genetic disease	32	41%	31%	N/D	13
16	2018	cohort	rWGS	infants; suspected genetic disease	42	43%	31%	26%	23
17	2018	cohort	rWES	acutely ill children with suspected genetic diseases	40	53%	30%	8%	16
18	2018	cohort	rWGS	children; PICU and cardiovascular ICU	24	42%	13%	N/D	9
19	2019	cohort	rWGS	4 months–18 years; PICU; suspected genetic diseases	38	48%	39%	8%	14
7	2019	cohort	rWGS	suspected genetic disease	195	21%	13%	N/D	21
20	2019	cases	urWGS	infants; suspected genetic disease	7	43%	43%	N/D	0.8
21	2019	cohort	rWES	<4 mo of age; ICU; hypotonia, seizures, metabolic, multiple congenital anomalies	50	54%	48%	N/D	5
22	2020	cohort	rWES	NICU & PICU; complex	130	48%	23%	N/D	3.8
23	2020	cohort	rWES	PICU; <6 years; new metabolic/neurologic disease	10	50%	30%	N/D	9.8
6, here	2019	RCT	rWGS	infants; disease of unknown etiology; within 96 h of admission	94	19%	24%	10%	11
			rWES		95	20%	20%	18%	11
			urWGS		24	46%	63%	25%	4.6
Weighted average, urWGS					35	49%	58%	25%	3.6
Weighted average, rWGS or rWES					894	37%	38%	16%	15.0

N/D, not done; RCT, randomized controlled trial.

of exome versus genome sequencing, nor rapid versus ultra-rapid sequencing. It was not surprising that clinicians perceived 93% of positive tests (those that identified a molecular diagnosis or incidental finding) as useful or very useful. However, the perception that 72% of negative tests had clinical utility was not anticipated. Discussions with clinicians revealed that they regarded genomic sequencing to have considerable negative predictive value (NPV). In many cases, clinicians specified disease genes for conditions they wished to have ruled in or out of the differential diagnoses. Clinicians appeared to employ informal Bayesian inferential reasoning, wherein negative genomic sequencing results updated the posterior probabilities of differential diagnoses. This requires further study since current laboratory validation techniques are not well designed to qualify the diagnostic NPV of genomic sequencing. In addition, the clinical validity of negative genomic sequencing results to inform management changes needs further study.

Prior to this study there was considerable evidence that rapid genomic sequencing had significant diagnostic utility in children in ICUs with suspected single-locus genetic diseases. Previous studies of genomic sequencing of ICU infants assessed observed clinical utility by combining rates of occurrence of individual changes in management (Table 3). Most prior studies were observational and examined diagnostic utility in cohorts. In previous studies, genomic sequencing occurred relatively late during hospitalization; testing was limited to children in whom genetic diseases were suspected; and patient selection was typically gated by clinical geneticists (Table 3). While such restricted use of genomic sequencing yielded high rates of diagnosis, it had the potential to delay or miss diagnoses in children with presentations that overlapped with common, non-genetic reasons for ICU

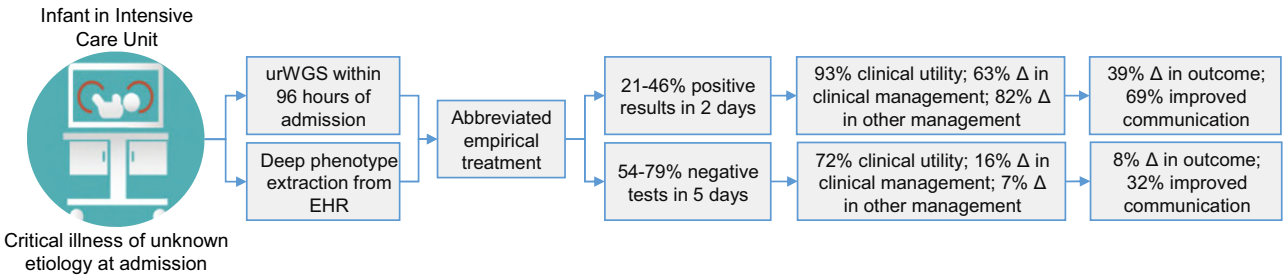

Figure 2. A Flow Diagram of NSIGHT2-Informed Proposed Best Practices and Expected Outcomes for Genome-Informed Intensive Care of Infants with Diseases of Unknown Etiology
Δ, change; urWGS, ultra-rapid whole-genome sequencing.

admission. Herein, 17 types of changes in acute management were evaluated, in addition to clinician perception of clinical utility. EHR review of 35% of infants with a perceived change in management revealed good agreement between clinician perceptions and EHR documentation. Overall, clinicians perceived that 28% of infants had changes in management as a result of diagnostic genomic sequencing. This value fell within the range of observed clinical utility in previous studies (Table 3). Clinicians did not report different change-in-management rates for rWGS versus rWES. In univariate analyses, however, change-in-management rates were significantly higher for urWGS and positive results. In multiple logistic regression analyses, positive results and shorter time to result were associated with higher change-in-management rates. Multiple logistic regression analyses also showed that change-in-management rates were significantly associated with clinician perceptions of utility. These findings suggest that physicians caring for infants in ICUs would find the highest value in a genomic sequencing test with immediate symptom-driven results and clear implications for care (Figure 2 and Table 2). Of note, negative tests changed management in 16% of infants, which was consistent with clinician perceptions that negative results were useful or very useful. Furthermore, this implies that the optimal indication for genomic sequencing should not focus entirely on infants with highest likelihood of genetic diseases. In other words, diagnostic yield is a poor proxy for the usefulness of genomic sequencing.

Prior to NSIGHT2, only five small studies had examined changes in outcomes of children in ICUs following genomic sequencing.[13,14,16,17,19] Three NSIGHT2 questions addressed perceived changes in outcome. Results of genomic sequencing were perceived to change outcomes in 15% of infants, a value that was within the range reported in those previous studies (Table 3). While the proportion of infants with a change in outcome did not differ significantly between genomic sequencing methods, positive tests were associated with more changes in outcome than negative tests. For many genetic diseases there is a dearth of organized knowledge of the natural history of disease or the effectiveness of therapies. As rapid diagnosis becomes commonplace, there will be an increasing need for clinical decision support for clinicians caring for infants in ICUs.[38] As previously noted, to achieve optimal reductions in morbidity and mortality, rapid genomic sequencing must be implemented within a comprehensive precision medicine delivery system.[38]

Finally, NSIGHT2 examined clinician perceptions of some potential harms of rapid genomic sequencing[35] (parental perceptions of the benefits and harms of rapid genomic sequencing in the NSIGHT2 study are reported in the accompanying paper[36]). When surveyed regarding their perceptions of increased stress or confusion among families or clinical staff as a result of genomic sequencing, clinicians reported this in only 3% of cases. This may reflect that NSIGHT2 was performed in a hospital with genomic medicine educational programs and training of ICU clinicians and genetic counselors about rapid genomic sequencing. Diagnostic genomic sequencing was felt to have improved communication with 41% of families regarding infant outcomes, expectations, and prognosis. Communication was improved more frequently in infants receiving urWGS and positive tests, probably because urWGS and positive results were available earlier, and positive tests decreased uncertainty. Another potential concern about genomic sequencing was that it would lead to numerous tests to confirm diagnoses and incidental findings or evaluate potential comorbidity. In practice, diagnostic genomic sequencing led to additional tests in 15% of infants, but cancellation of tests in 21% of infants.

NSIGHT2 had several weaknesses. The foremost was insufficient study size to test some meaningful hypotheses. Unfortunately, the original intent—enrollment of 1,000 infants—exceeded the budget of conventional NIH grants. Specifically, NSIGHT2 lacked power to detect differences in the clinical utility of rWGS and rWES, or to stratify results by organ system or disease type. When NSIGHT2 started, urWGS could not be scaled for performance in all randomized infants and was reserved for an unmatched group of very sick infants. Thanks to technological improvements, such as the NovaSeq 6000 instrument and S1 flowcell, we now perform urWGS on a majority of infants receiving genomic sequencing.[20] Given the study design, however, it cannot be definitively determined whether the greater rate of change in management and perceived improvement in communication associated with urWGS were due to shorter time to result or selection bias in infants receiving urWGS. Another weakness was that changes in

management and outcome in each infant typically reflected the perception of a single clinician. A more robust methodology might have been to have had several clinicians complete the survey for each infant are reporting of concordance of clinician perception.

In conclusion, when used broadly as a first-tier test for infants in ICUs with diseases of unknown etiology, rapid genomic sequencing was associated with clinician-reported utility in three quarters of cases, changes in management in more than a quarter, and perceived improved communication with 40% of families (Figure 2). It seldom led to confusion or distress. Rapid genomic sequencing was associated with changes in outcomes in 15% of infants. Physician-perceived benefits of rapid genomic sequencing were not limited to those receiving positive results. These results, together with the prior literature, support general use of rapid genomic sequencing among infants in ICUs.

Data and Material Availability

All data associated with this study are present in the paper, Supplemental Data, or are available at the Longitudinal Pediatric Data Resource (LPDR) under a data use agreement and subject to the limitations of the informed consent documents for each subject (NBSTRN: nbs000003.v1.p).

Supplemental Data

Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2020.10.003.

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Web Resources

ClinicalTrials.gov, https://clinicaltrials.gov
LPDR, http://www.nbscn.org/longitudinal-pediatric-data-resource.htm
OMIM, https://www.omim.org/
R statistical software, https://www.r-project.org/

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