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SUMMARY WITH CRITICAL APPRAISAL

Rapid Genome-wide Testing: A Review of Clinical Utility, Cost-Effectiveness, and Guidelines

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Abbreviations

CRD	Centre for Reviews and Dissemination
MeSH	Medical Subject Headings
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	randomized controlled trial

Context and Policy Issues

Following the elucidation of the molecular structure of DNA in the 1950s with the work of Rosalind Franklin, Maurice Wilkins, Francis Crick, and James Watson, the field of medical genetics and genomics has undergone remarkable advancements in technology.[1] The first DNA sequencing techniques were developed in the 1970s,[2,3] which, in combination with advancements in computing, enabled the launch of the Human Genome Project in 1990. This international scientific project successfully mapped out a reference human genome over the course of 15 years with a budget of approximately three billion dollars,[4] providing a foundation for medical genetics in the 21st century.

More recently, next-generation sequencing techniques have exponentially decreased costs and improved the accuracy of various genetic screening tests.[5] These tests have been incorporated into clinical practices to aid in the identification and diagnosis of various genetic conditions. Genetic samples from individuals with illnesses of unknown (or suspected genetic) etiology can be examined using a targeted panel of genes to determine whether clinical observations are attributable to known genetic conditions with associated sequences.[6]

Congenital malformation, deformations, and chromosomal abnormalities are the leading cause of mortality for infants less than one year of age in many developed countries, including Canada.[7-9] A proper diagnosis (confirmed by genetic testing) may lead to changes in the clinical management of individuals; however, these tests traditionally have a typical turn-around time of weeks to months. Although this timeframe may be appropriate in certain circumstances, it is possible that serious patient deterioration or harm may occur prior to the establishment an underlying diagnosis and the initiation of appropriate treatment, especially for those presenting to intensive care units.[10] Rapid genome-wide tests, which are capable of providing a result as quickly as one week after sample collection,[11] may provide benefit over traditional genome-wide tests with standard turnaround times as they enable quicker access to precision medicine interventions.[12]

The objective of the current report is to evaluate the clinical utility, cost-effectiveness, and evidence-based guidelines regarding the provision of rapid turnaround for genome-wide testing for patients in intensive care. This report expands upon a previously completed CADTH report (summary of abstracts).[13]

Research Questions

1. What is the clinical utility of providing rapid turnaround for genome-wide testing for patients in intensive care?
2. What is the cost-effectiveness of providing rapid turnaround for genome-wide testing for patients in intensive care?
3. What are the evidence-based guidelines of providing rapid turnaround for genome-wide testing for patients in intensive care?

Key Findings

Two relevant clinical studies, including one randomized controlled trial and one non-randomized study, were identified regarding the clinical utility of providing rapid turnaround for genome-wide testing for patients in intensive care.

Evidence of limited quality demonstrated that genome-wide tests with a rapid turnaround time significantly decreased the time to diagnosis for infants in intensive care compared to standard genetics tests with a routine turnaround time. There were mixed results regarding how rapid tests may have ultimately affected medical management. One study reported no significant differences in the rates of change in medical management following the interpretation of the results from a rapid genetic test plus standard tests versus standard genetic tests alone. The second study observed a significantly higher number of cases with changes to medical management following a test result in a cohort that received a rapid test versus cohorts that received genetic tests with a standard turnaround time. Neither of the included studies noted significant differences in rates of mortality between infants who received rapid genome-wide testing or those who received genome-wide testing with routine turnaround time.

No evidence regarding the cost-effectiveness of providing rapid turnaround for genome-wide testing for patients in intensive care was identified. Additionally, no relevant evidence-based guidelines were identified. The limitations of the included studies (e.g., their open-label nature, lack of reporting on patients lost to follow-up) should be considered when interpreting the findings of this report.

Methods

Literature Search Methods

This report makes use of a literature search developed for a previous CADTH report.[13] A limited literature search was conducted by an information specialist on key resources including PubMed, the Cochrane Library, the University of York Centre for Reviews and Dissemination (CRD) databases, the websites of Canadian and major international health technology agencies, as well as a focused Internet search. The search strategy was comprised of both controlled vocabulary, such as the National Library of Medicine's MeSH (Medical Subject Headings), and keywords. The main search concepts were rapid genome testing and intensive care units. No filters were applied to limit the retrieval by study type. Where possible, retrieval was limited to the human population. The search was also limited to English language documents published between January 1, 2014 and August 13, 2019.

Selection Criteria and Methods

One reviewer screened citations and selected studies. In the first level of screening, titles and abstracts were reviewed and potentially relevant articles were retrieved and assessed for inclusion. The final selection of full-text articles was based on the inclusion criteria presented in Table 1.

Table 1: Selection Criteria

Population	Patients of all ages in an intensive care setting (i.e., neonatal, pediatric, or adults)
Intervention	Rapid genome-wide testing (e.g., rapid/expedited/express whole exome sequencing, broad panel of multiple genes [e.g., neonatal crisis panel]) - Rapid or expedited turnaround time = 1 to 4 weeks
Comparator	No testing; genome-wide testing with routine turnaround time (i.e., 6 to 12 weeks)
Outcomes	Q1: Clinical utility, clinical outcomes (e.g., mortality, change in active patient management) Q2: Cost-effectiveness Q3: Guidelines
Study Designs	Health technology assessments, systematic reviews, meta-analyses, randomized controlled trials, non-randomized studies, economic evaluations, and evidence-based guidelines

Exclusion Criteria

Articles were excluded if they did not meet the selection criteria outlined in Table 1, they were duplicate publications, or were published prior to 2014. Studies on the use of rapid genome-wide testing in pre-natal patients (i.e., in utero) were excluded. Finally, guidelines with unclear methodology were also excluded.

Critical Appraisal of Individual Studies

One reviewer critically appraised the clinical studies using the Downs and Black checklist.[14] Summary scores were not calculated for the included studies; rather, the strengths and limitations of each included study were described narratively.

Summary of Evidence

Quantity of Research Available

A total of 364 citations were identified in the literature search. Following screening of titles and abstracts, 358 citations were excluded and six potentially relevant reports from the electronic search were retrieved for full-text review. In addition, four potentially relevant publications were retrieved from the grey literature search for full-text review. Of these 10 potentially relevant articles, eight publications were excluded for various reasons, while two publications met the inclusion criteria and was included in this report. These comprised one randomized controlled trial[15] (RCT) and one non-randomized study.[16] Appendix 1 presents the PRISMA[17] flowchart of the study selection. Additional references of potential interest are provided in Appendix 5.

Summary of Study Characteristics

One relevant RCT[15] and one non-randomized study[16] were identified for inclusion in this review. No relevant health technology assessments, systematic reviews, meta-analyses, economic evaluations, or evidence-based guidelines were identified. Detailed characteristics are available in Appendix 2, Table 2.

Study Design

Two studies[15,16] were included regarding the clinical utility of providing rapid turnaround for genome-wide testing for patients in intensive care. The study by Petrikin et al.[15] was a

partially blinded (clinicians and parents were blinded until ten days post-randomization to minimize parental anxiety and allow consideration for crossover to rapid whole-genome sequencing), single-centre, pragmatic RCT. Participant recruitment occurred between October 2014 and June 2016. The included non-randomized study[16] was a retrospective, single-centre cohort study that included data collected between December 2011 and January 2017.

Country of Origin

The included clinical studies[15,16] were conducted in the United States.

Patient Population

The RCT[15] recruited infants (<4 months of age) in the neonatal or pediatric intensive care units with illness of unknown etiology and features suggestive of a genetic disease. The study excluded infants with a previously confirmed genetic diagnosis that explained the clinical condition or those with features indicative of a chromosomal aberration. A total of 65 infants were randomized and included in the analysis. The mean age of infants at time of enrollment was 22.4 days (range of 1 day to 101 days). The proportion of female participants was 40% (57% male, 3% undetermined).

The non-randomized study[16] included data from unrelated infants (≤100 days of age at time of testing) who received exome sequencing in neonatal and pediatric intensive care units between December 2011 and January 2017. Data from 278 consecutive infants were included in the analysis. The mean age of participating infants was 28.5 days and the proportion of female participants was 45% (55% male).

Interventions and Comparators

The intervention in the RCT[15] was genetic testing with rapid whole-genome sequencing plus standard diagnostic tests for genetic diseases, compared to standard diagnostic tests alone. Rapid whole-genome sequencing was performed on infant-parent trios (when available) with Illumina HiSeq instruments. The results of the rapid test were confirmed using Sanger sequencing prior to the initiation of change in medical management; however, confirmatory tests were not required in cases where life-threatening progression was imminently likely, and clinical management could be altered based on the rapid test alone. Standard diagnostic tests for genetic diseases included all postnatal tests that could be ordered through the electronic medical record, based on physician clinical judgment. These tests included biochemical and immunologic testing for genetic diseases, array comparative genomic hybridization, fluorescence in situ hybridization, high resolution chromosome analysis, Sanger sequencing, non-expedited proband targeted next-generation sequencing gene panels, non-expedited proband whole-exome sequencing, non-expedited proband whole-genome sequencing, methylation studies, gene deletion or duplication assays, and Kansas or Missouri state newborn screening. Following unblinding at day 10, participants in the standard tests group were considered for compassionate crossover to the rapid whole-genome sequencing group (which occurred in five out of 33 individuals).

The intervention in the non-randomized study[16] was critical trio exome sequencing (a rapid genomic assay), compared to proband exome sequencing or trio exome sequencing offered at Baylor Genetics. Exome data were interpreted according to the guidelines produced by the American College of Medical Genetics and Genomics and the variant interpretation guidelines of Baylor Genetics.

Outcomes

The primary outcome in the RCT[15] was the proportion of infants who received a genetic diagnosis within 28 days. Secondary outcomes included the proportion of infants with a diagnosis by day of life 28, the total number of diagnoses made, the clinical utility of diagnoses (the proportion of infants who had a change in clinical management resulting from their diagnosis), the proportion of infants with a diagnosis before discharge, the mortality rate at 180 days, and the median age at death.

The non-randomized study[16] examined diagnostic yield (defined as the proportion of individuals who received a diagnosis following referral to genetic testing), intensive care unit length of stay, mortality rates at 120 days and five years, median age at diagnosis, proportion of infants with a diagnosis before discharge, and clinical utility (defined as the proportion of individuals who had changes in their medical management following the interpretation of genetic test results).

Summary of Critical Appraisal

Additional details regarding the strengths and limitations of the included publications are provided in Appendix 3, Table 3.

The included clinical studies[15,16] had clearly described objectives, interventions, controls, patient recruitment methodology, inclusion and exclusion criteria, clinical outcomes, and main findings. Details on baseline participant characteristics (e.g., age, sex, ethnicity, birth characteristics) were included and were tested for statistically significant differences between groups at baseline. Although treatment groups were relatively balanced with regards to these characteristics, there were some instances where significant differences were detected between intervention groups. For example, participants in the control group of the Petrikin et al.[15] RCT had fewer cardiovascular findings than those tested with rapid genome sequencing, which may have affected the likelihood for genetic disease. In other words, some between-group differences (e.g., in number of diagnoses) may have been due to systematic differences in patients allocated to each group and not due to differences in the interventions (e.g., ability of the rapid vs. standard tests to detect genetic conditions). Additionally, the genetic pathologies of participants in both studies[15,16] were highly heterogenous; it was unclear how this heterogeneity may have influenced the findings. Although the RCT[15] was partially blinded, the clinicians and participants were unblinded at the time of outcome assessment in both studies.[15,16] Additionally, intervention assignment was done at the clinician's discretion based on the diagnostic tests available at the time in the non-randomized study.[16] As a result, there was a risk for bias in either direction depending on the perceptions and expectations of clinicians and outcome assessors. The authors of the RCT[15] conducted a sample size calculation prior to patient recruitment and proposed a sample size of 1,000 participants (500 in each group); however, the study was terminated early due to a loss of equipoise (after 65 participants had been randomized), resulting in loss in power for the secondary end-points (e.g., clinical utility, mortality at 180 days, age of death). No power calculation was performed in the non-randomized study.[16] Due to the nature of the intervention, compliance with the assigned treatment appears to be reliable in both studies. The length of follow-up was consistent between the treatment and control groups in the RCT[15] (180 days after randomization), but the follow-up length and methods for handling patients lost to follow-up were unclear in the non-randomized study.[16] Actual probability values (*P*-values) and estimates of random variability (e.g., standard errors) were reported in both studies,[15,16] increasing the strength of reporting. Potential conflicts of interest were declared in both studies. The authors of the non-randomized

study[16] disclosed ties with industry (several authors were affiliated with the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from the clinical exome sequencing offered by Baylor Genetics; one author was a member of the Scientific Advisory Board of Veritas Genetics). The authors of both studies reported on their sources of funding (which were considered unlikely to have influenced the findings of the studies).

Study participants, care providers, and health care settings appeared to be representative of the "real-world" in both included clinical studies,[15,16] increasing their external validity. Additionally, the use of a pragmatic design in the RCT[15] that allowed participant cross-over based on clinician judgement (while conducting the analysis based on intention to treat principles) may more accurately reflect clinical circumstances than a trial conducted without this flexibility. However, the studies[15,16] were conducted at single centres in the United States, and the generalizability of the findings to other centres or countries is not clear.

Summary of Findings

The overall findings of the included study are summarized below. A detailed summary of the main findings is available in Appendix 4, Table 4.

Clinical Utility of Providing Rapid Turnaround for Genome-wide Testing

Clinical Utility

Information on the clinical utility of providing rapid turnaround for genome-wide testing was available from one RCT[15] and one non-randomized-study.[16]

The authors of the RCT[15] reported similar number of total diagnoses made in the rapid whole-genome sequencing plus standard diagnostic tests for genetic diseases versus those given standard diagnostic tests alone (41% vs 24%; *P* = 0.19); however, infants in the rapid whole-genome sequencing plus standard diagnostic tests group were significantly more likely to have a diagnosis within 28 days of study enrollment (31% vs. 3%; *P* = 0.003) and to have a diagnosis by day of life 28 (32% vs. 0%, *P* = 0.004). Overall, the clinical utility of the diagnoses did not significantly differ between treatment groups (41% vs. 21%; *P* = 0.11).

The findings of the non-randomized study[16] suggested that infants given critical trio exome sequencing (the “rapid” genome-wide test) were more likely to receive a diagnosis compared to those who received proband exome or trio exome sequencing (*P* = 0.01). The median age at time of diagnosis was younger in the critical exome group (*P* = 0.02) and these participants were more likely to receive a diagnosis before discharge than those in the proband exome or trio exome groups (*P* = <0.001). These diagnoses affected medical management in 71.9% of patients in the critical trio exome group, which was significantly higher than those in the proband exome or trio exome groups, in which medical management was affected in 45.6% and 33.3% of infants, respectively (*P* = 0.01).

Mortality

Evidence regarding the effectiveness of providing rapid turnaround for genome-wide testing with respect to mortality was available from one RCT[15] and one non-randomized-study.[16]

The RCT[15] did not detect any statistically significant difference between infants given rapid whole-genome sequencing plus standard diagnostic tests for genetic diseases versus those given standard diagnostic tests alone with respect to rate of mortality at 180 days (13% vs.

12%; P = 0.91). Similarly, there were no significant differences between the groups for median age of death.

The authors of the non-randomized study[16] reported no significant differences in mortality rates at 120 days or five years in infants who received critical trio exome sequencing (a rapid genomic assay) versus infants who received genetic testing with proband exome or trio exome sequencing. This finding was observed for both diagnosed and undiagnosed study participants.

Cost-Effectiveness of Providing Rapid Turnaround for Genome-wide Testing

No relevant evidence regarding the cost-effectiveness of providing rapid turnaround for genome-wide testing for patients in intensive care was identified; therefore, no summary can be provided.

Guidelines

No relevant evidence-based guidelines regarding the provision of rapid turnaround for genome-wide testing for patients in intensive care were identified; therefore, no summary can be provided.

Limitations

A number of limitations were identified in the critical appraisal (Appendix 3, Table 3), however, additional limitations exist.

The quantity of identified relevant literature was relatively low. The clinical utility findings were drawn from one partially blinded RCT[15] and one non-randomized retrospective cohort study. Both studies may be subject to selection bias because participants were either not randomized to treatment groups or unblinded clinicians had the opportunity to crossover participants between intervention groups. It is possible that the clinician perceptions and expectations may have played a role in patient allocation and thereby introduced systematic differences between the groups that were unrelated to the intervention(s) received.

The included studies[15,16] were specific to neonate or infant (less than four months of age) populations; thus, the clinical utility of rapid genome-wide testing for older populations is unknown.

No evidence regarding the cost-effectiveness of providing rapid turnaround for genome-wide testing for patients in intensive care was identified. Additionally, no evidence-based guidelines for providing rapid turnaround for genome-wide testing for patients in intensive care were identified.

The applicability of the evidence to Canadian settings is unclear as the clinical studies[15,16] were conducted in the United States. Any differences in the utilization of genetic testing (rapid or non-rapid) for patients in intensive care in the United States and Canada may affect the generalizability of the findings.

Conclusions and Implications for Decision or Policy Making

This review was comprised of one RCT[15] and one non-randomized study[16] regarding the clinical utility of providing rapid turnaround for genome-wide testing for patients in intensive care. No relevant cost-effectiveness literature or evidence-based guidelines were identified.

Evidence of limited quality suggested that rapid genome-wide tests may decrease the time to diagnosis for infants in intensive care; however, the included studies had mixed findings regarding the clinical utility of rapid tests. One study[15] reported no significant differences in the clinical utility of rapid whole-genome sequencing plus standard tests versus standard genetic tests alone. The second study[16] observed significantly higher rates of changes in medical management in a cohort of infants who received a rapid genome test versus those that received genetic tests with a standard turnaround time. Despite these differences however, neither of the included studies[15,16] noted significant differences in rates of mortality between infants who received rapid genome-wide testing or those who received genome-wide testing with routine turnaround time.

Although this review was intended as an upgrade to a previously published report,[13] two studies[5,12] included in the predecessor report were excluded in this review following full-text assessment. These studies were excluded as they did not include relevant comparators; rapid genome-wide testing was not directly compared with standard genome-testing or no genetic testing.

The limitations of the included studies[15,16] and of this report should be considered when interpreting the results. The findings highlighted in this review come with a high degree of uncertainty. Further research investigating the clinical utility and cost-effectiveness of rapid genome-wide testing for patients in an intensive care setting, particularly in non-neonate populations, would help reduce this uncertainty.

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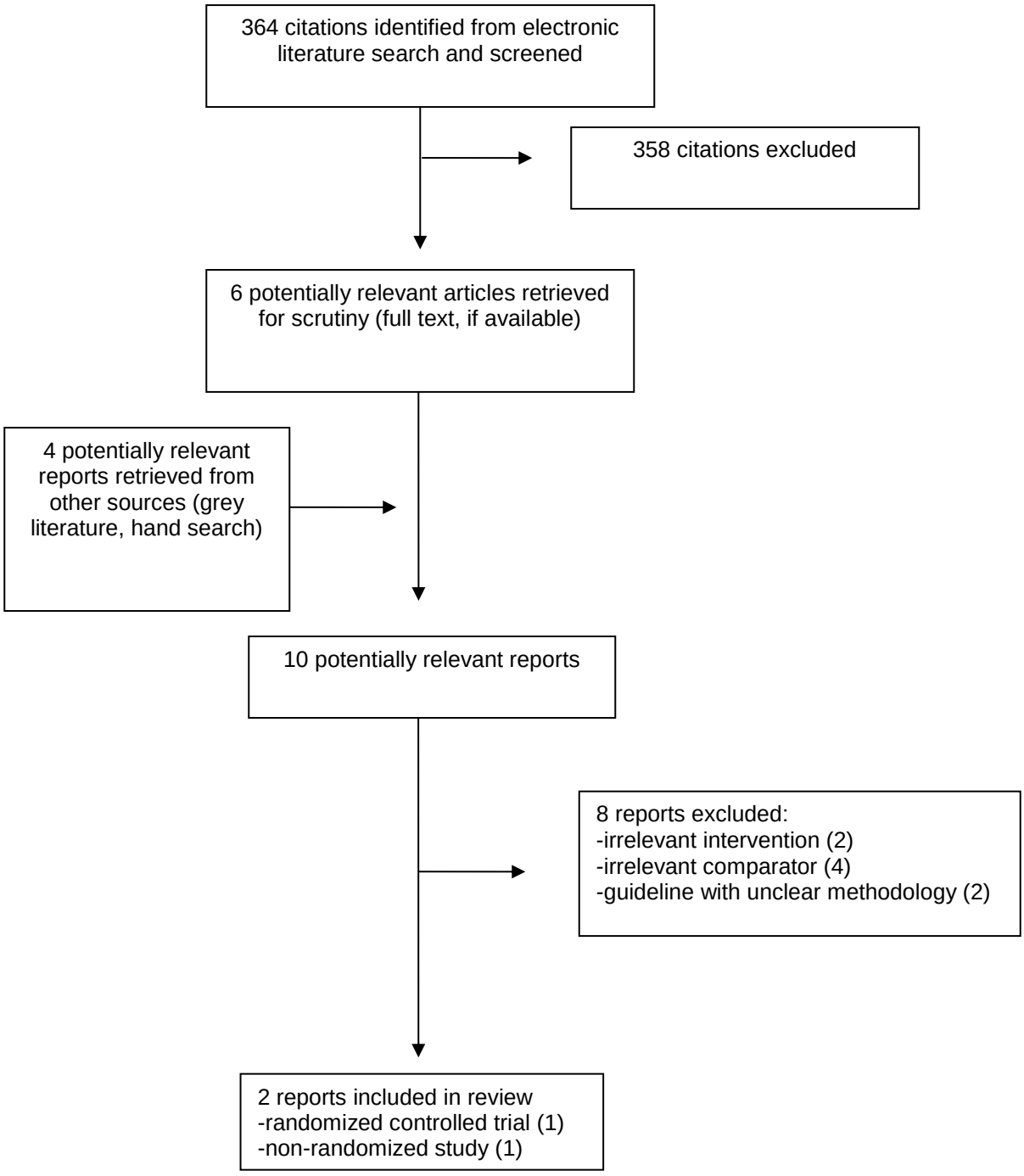
Appendix 1: Selection of Included Studies



Appendix 2: Characteristics of Included Publications

Table 2: Characteristics of Included Primary Clinical Studies

Study Citation, Country, Funding Source	Study Design, Objective, and Setting	Participant Characteristics	Intervention and Comparator(s)	Clinical Outcomes, Length of Follow-Up
Randomized Controlled Trial				
Petrikin et al., 2018[15] United States **Funding source**: A grant from the National Human Genome Research Institute and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (grant U19HD077693)	**Study design**: Partially blinded (clinicians and parents were blinded until day ten post-randomization to minimize parental anxiety and allow consideration for crossover to rWGS), single-centre, pragmatic RCT (using a 1:1 ratio). **Objective**: To determine whether the addition of rWGS to standard genetic tests decreased the time to diagnosis in infants with suspected genetic diseases. **Setting**: Recruitment occurred between October 2014 and June 2016 in the neonatal or pediatric intensive care units of a tertiary referral children's hospital (Children's Mercy) in Kansas City, United States.	**Inclusion criteria**: Infants (<4 months of age) in the neonatal or pediatric intensive care units with illness of unknown etiology and one of the following criteria: (1) an order for genetic test or genetic consult; (2) a major structural congenital anomaly or at least 3 minor anomalies; (3) an abnormal laboratory test indicating a genetic disease; (4) an abnormal response to standard therapy for a major underlying condition. **Excluded**: Individuals with a previously confirmed genetic diagnosis that explained the clinical condition or those with features pathognomonic for a chromosomal aberration. **Number of participants**: 65 (32 in the rWGS group; 33 in the standard tests alone group). 5 individuals crossed-over to rWGS following unblinding after 10 days. **Mean age, days (range)**: 22.8 (1 to 101) in the rWGS group; 22.0 (1 to 80) in the standard tests alone group. **Sex**: 47% female in the rWGS group (50% male, 3% undetermined); 33% female in the standard tests alone group (64% male, 3% undetermined).	**Intervention**: Rapid whole-genome sequencing (rWGS) plus standard diagnostic tests. rWGS were performed using Illumina HiSeq instruments. Standard diagnostic tests included all postnatal diagnostic tests that could be ordered through the electronic medical record. These standard tests included biochemical and immunologic testing for genetic diseases, array comparative genomic hybridization, fluorescence in situ hybridization, high resolution chromosome analysis, Sanger sequencing, non-expedited proband targeted next-generation sequencing gene panels, non-expedited proband whole-exome sequencing, non-expedited proband whole-genome sequencing, methylation studies, gene deletion or duplication assays, and Kansas or Missouri state newborn screening. **Comparator**: Genetic testing with standard diagnostic tests alone (as described above).	**Primary Outcome**: - Diagnosis within 28 days of enrollment **Secondary Outcomes**: - Diagnosis by day of life 28 - Total diagnoses - Clinical utility of diagnoses - Days of life at time of hospital discharge - Number of diagnoses before discharge - Mortality at 180 days - Age of death **Follow-up**: 6 months

Table 2: Characteristics of Included Primary Clinical Studies

Study Citation, Country, Funding Source	Study Design, Objective, and Setting	Participant Characteristics	Intervention and Comparator(s)	Clinical Outcomes, Length of Follow-Up
Non-Randomized Study				
Meng et al., 2017[16] United States **Funding source**: Financial support was received from March of Dimes (#6-FY16-176) and the National Institutes of Health (T32GM007526-39).	**Study design**: Retrospective, single-centre cohort study **Objective**: To evaluate the clinical utility of exome sequencing (probrand exome, trio exome, and critical trio exome) for unrelated infants in neonatal and pediatric intensive care units. **Setting**: Data from infants who received exome sequencing between December 2011 and January 2017 at the neonatal and pediatric intensive care units of the Texas Children's Hospital was included in the study.	**Inclusion criteria**: Unrelated infants (≤100 days of age at time of testing) who were referred from the Texas Children's Hospital for exome sequencing. **Excluded**: No specific exclusion criteria were applied. **Number of participants**: 278 (63 in the critical trio exome cohort; 178 in the proband exome cohort; 37 in the trio exome cohort). **Mean age, days (SE)**: 28.5 (1.7) in the entire population; 22.7 (3.9) in the critical trio exome cohort; 29.0 (2.2) in the proband exome cohort; 31.5 (3.9) in the trio exome cohort. **Sex**: 45% female in the entire population (55% male).	**Intervention**: Exome sequencing using critical trio exome (available since April 2015), a rapid test offered by Baylor Genetics. **Comparators**: Sequencing using proband exome (available since December 2011) or trio exome (available since October 2014). Exome data were interpreted according to ACMG guidelines and variant interpretation guidelines of Baylor Genetics.	**Outcomes**: - Diagnostic yield - Death rates (120-day and 5-year) - Turnaround time - Molecular findings - Patient age at diagnosis - Impact on medical management **Follow-up**: NR

ACMG = American College of Medical Genetics; NR = not reported; RCT = randomized controlled trial; rWGS = rapid whole-genome sequencing; SE = standard error.

Appendix 3: Critical Appraisal of Included Publications

Table 3: Strengths and Limitations of Clinical Studies using the Downs and Black Checklist[14]

Strengths	Limitations
Randomized Controlled Trial	
Petrikin et al., 2018[15]	
• The objectives, interventions, controls, and main outcomes were clearly described • Detailed methodology on patient recruitment and assessment of inclusion/exclusion criteria was included • Population characteristics (e.g., age, sex, ethnicity, birth characteristics, primary system involved by disease) were clearly described and were tested for statistically significant differences (participants in the control arm had fewer cardiovascular findings) • Compliance with the assigned intervention was reliable • Outcome measures were valid and reliable • The major findings of the study were presented in tabular form and clearly described • There were no patients lost to follow-up • Length of follow-up was the same for all study participants • Estimates of random variability (e.g., standard errors) and actual probability values (*P*-values) were reported • Study participants, care providers, and setting appeared to be representative of the population and care setting of interest • Sources of funding were disclosed and were unlikely to have had an effect on the findings of the study • The authors declared that they had no potential conflicts of interest	• The study was partially blinded (clinicians and parents were blinded until day ten post-randomization to minimize parental anxiety and allow consideration for crossover to rWGS), with unblinding occurring before outcomes were assessed • The genetic pathology of participants allocated to intervention groups was highly heterogeneous; uncontrolled factors may have contributed to the findings of the study • Several patients (N = 5/33) were crossed over from the control group to the rWGS + standard care group for compassionate reasons. These individuals were included in the control group for statistical analyses (which used intention-to-treat principles), despite receiving both interventions • A power calculation was performed; however, the sample size proposed in the calculation (1000 total, 500 in each group) was not reached (65 individuals were recruited in total) •
Non-Randomized Study	
Meng et al., 2017[16]	
• The objectives, interventions, controls, and main outcomes were clearly described • Detailed methodology on patient recruitment and assessment of inclusion/exclusion criteria was included • Population characteristics (e.g., age, sex) were clearly described and were tested for statistically significant differences at baseline (there were no significant differences) • Compliance with the assigned intervention was reliable • Outcome measures were valid and reliable • The major findings of the study were presented in tabular form and clearly described • Estimates of random variability (e.g., standard errors) and actual probability values (*P*-values) were reported	• Details on the source of data and methods of data collection were lacking • It was unclear how patients lost to follow-up were handled, which is especially concerning for outcomes with relatively long follow-up durations (e.g., 5-year death rate) • This was an open-label study with no blinding of study participants or outcome assessors • Intervention assignment was not done at random (assignment was likely done at the clinician's discretion based on the clinical circumstances and the tests that were available at the time), and the phenotypic abnormalities and underlying genetic causes were heterogeneous within the study sample; therefore, a number of unmeasured and/or uncontrolled factors may have contributed to the findings of the study

Table 3: Strengths and Limitations of Clinical Studies using the Downs and Black Checklist[14]

Strengths	Limitations
• Study participants, care providers, and setting appeared to be representative of the population and care setting of interest • Sources of funding were disclosed and were unlikely to have had an effect on the findings of the study	• Study subjects in the intervention and control groups were not recruited over the same period of time (the sequencing tests were not all available at the start of the study; therefore, participants from early in the study only had access to one intervention while participants recruited more recently had access to all three) • A power calculation was not performed to determine the required sample size • Conflicts of interest were disclosed (several authors were affiliated with the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from the clinical exome sequencing offered by Baylor Genetics; one author was a member of the Scientific Advisory Board of Veritas Genetics) • Single-centre study (conducted in the United States); the generalizability to the Canadian setting is unclear

N = number of patients; rWGS = rapid whole-genome sequencing.

Appendix 4: Main Study Findings and Authors' Conclusions

Table 4: Summary of Findings of Included Primary Clinical Studies

<table>
<tr><th>Main Study Findings</th><th>Authors' Conclusion</th></tr>
<tr><td colspan="2">Randomized Controlled Trial</td></tr>
<tr><td colspan="2">Petrikin et al., 2018[15]</td></tr>
<tr><td>A partially blinded, single-centre, pragmatic RCT that sought to determine whether the addition of rWGS to standard genetic tests decreased the time to diagnosis in infants with suspected genetic diseases.

Comparison of rapid whole-genome sequencing plus standard testing (rWGS + ST) and standard testing alone (ST; included the 5 participants that crossed over and also received rWGS) with respect to several clinical outcomes
<table>
<tr><th rowspan="2">Outcome</th><th colspan="2">Intervention group</th><th rowspan="2">Statistical significance (P-value)</th></tr>
<tr><th>rWGS + ST (N = 32)</th><th>ST (N = 33)</th></tr>
<tr><td>Diagnosis within 28 days of enrollment, N (%)</td><td>10 (31%)</td><td>1 (3%)</td><td>0.003</td></tr>
<tr><td>Diagnosis by day of life 28, N (%)</td><td>7 (32%)</td><td>0 (0%)</td><td>0.004</td></tr>
<tr><td>Total diagnoses, N (%)</td><td>13 (41%)</td><td>7 (21%)</td><td>0.19</td></tr>
<tr><td>Clinical utility of diagnoses, N (%)</td><td>13 (41%)</td><td>7 (21%)</td><td>0.11</td></tr>
<tr><td>Mean age at hospital discharge, days (range)</td><td>66.3 (3 to 456)</td><td>68.5 (4 to 341)</td><td>0.91</td></tr>
<tr><td>Diagnosis before discharge, N (%)</td><td>9 (28%)</td><td>3 (9%)</td><td>0.06</td></tr>
<tr><td>Mortality at 180 days, N (%)</td><td>4 (13%)</td><td>4 (12%)</td><td>NR</td></tr>
<tr><td>Median age at death, days (range)</td><td>62 (14 to 228)</td><td>173 (4 to 341)</td><td>0.93</td></tr>
</table>
N = number of patients; NR = not reported; rWGS + ST = rapid whole-genome sequencing plus standard testing; ST = standard testing alone.

Credit: Adapted from Petrikin JE, Cakici JA, Clark MM, et al. NPJ Genomic Medicine: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5807510/ CC by 4.0: https://creativecommons.org/licenses/by/4.0/</td><td>"Among infants with suspected genetic diseases in a regional NICU or PICU, the addition of rWGS decreased the time to diagnosis. Since genetic diseases are among the leading cause of death in the NICU and PICU, as well as overall infant mortality, implementation of rWGS is likely to have broad implications for the practice of neonatology."[15] (p7)</td></tr>
<tr><td colspan="2">Non-Randomized Study</td></tr>
<tr><td colspan="2">Meng et al., 2017[16]</td></tr>
<tr><td>A retrospective, single-centre cohort study that investigated the diagnostic yield and use of clinical exome sequencing in critically ill infants.

Comparison of proband exome (PE; N = 176), trio exome (TE; N = 39), and critical trio exome (CTE; N = 63; the rapid genomic assay) sequencing techniques with respect to several clinical outcomes.

Proportion of individuals who received an exome sequencing diagnosis:
• PE = 32.4%; TE = 33.3%; CTE = 50.8%
• Odds ratio (95% CI) = 2.14 (1.21 to 3.78)*
• Between-group (PE and TE versus CTE) statistical significance: P = 0.01

Median turnaround time, days (SE):
• PE = 95.0 (1.5); TE = 51.1 (3.2); CTE = 13.0 (0.4)
• Between-group (PE and TE versus CTE) statistical significance: P < 0.001

Median ICU stay length, days (SE):
• Diagnosed individuals
  ○ PE = 28.0 (6.3); TE = 32.0 (14.3); CTE = 42.5 (10.2)
  ○ Between-group (PE and TE versus CTE) statistical significance: P = 0.11
• Undiagnosed individuals
  ○ PE = 41.0 (5.8); TE = 35.0 (6.9); CTE = 31.0 (13.4)</td><td>"Our study provides strong evidence that clinical exome sequencing uncovers monogenic disorders in a significant number of infants in NICUs and [PICUs] who are suspected to have genetic disorders, significantly affecting the medical care of more than half of infants who receive diagnoses."[16] (p9)</td></tr>
</table>

Table 4: Summary of Findings of Included Primary Clinical Studies

Main Study Findings	Authors' Conclusion
○ Between-group (PE and TE versus CTE) statistical significance: *P* = 0.83 **5-year death rate**: • Diagnosed individuals ○ PE = 47.4%; TE = 15.4%; CTE = 31.3% ○ Odds ratio (95% CI) = 0.64 (0.27 to 1.56)* ○ Between-group (PE and TE versus CTE) statistical significance: *P* = 0.38 • Undiagnosed individuals ○ PE = 25.6%; TE = 12.0%; CTE = 28.6% ○ Odds ratio (95% CI) = 1.32 (0.53 to 3.27)* ○ Between-group (PE and TE versus CTE) statistical significance: *P* = 0.63 **120-day death rate**: • Diagnosed individuals ○ PE = 31.6%; TE = 15.4%; CTE = 31.3% ○ Odds ratio (95% CI) = 1.14 (0.46 to 2.82)* ○ Between-group (PE and TE versus CTE) statistical significance: *P* = 0.82 • Undiagnosed individuals ○ PE = 17.9%; TE = 4.0%; CTE = 21.4% ○ Odds ratio (95% CI) = 1.49 (0.54 to 4.09)* ○ Between-group (PE and TE versus CTE) statistical significance: *P* = 0.58 **Median age at diagnosis, days (SE)**: • PE = 116.5 (27.4); TE = 78.0 (103.1); CTE = 33.1 (5.6) • Between-group (PE and TE versus CTE) statistical significance: *P* = 0.002 **Proportion of diagnosed individuals who received their diagnosis before discharge**: • PE = 22.8%; TE = 30.8%; CTE = 65.6% • Odds ratio (95% CI) = 5.95 (2.39 to 14.81)* • Between-group (PE and TE versus CTE) statistical significance: *P* < 0.001 **Proportion of diagnosed individuals who had their medical management affected**: • PE = 45.6%; TE = 33.3%; CTE = 71.9% • Odds ratio (95% CI) = 3.41 (1.38 to 8.42)* • Between-group (PE and TE versus CTE) statistical significance: *P* = 0.01 ***Note:** Odds ratios were expressed comparing CTE versus other sequencing techniques (PE and TE)	

CI = confidence interval; CTE = critical trio exome; ICU = intensive care unit; N = number of patients; NICU =neonatal intensive care unit; PE = proband exome; PICU = pediatric intensive care unit; RCT = randomized controlled trial; rWGS = rapid whole-genome sequencing; SE = standard error; TE = trio exome.

Appendix 5: Additional References of Potential Interest

Previous CADTH Reports

Next generation DNA sequencing: a review of the cost effectiveness and guidelines (*Rapid Response Report: Summary with Critical Appraisal*). Ottawa (ON): CADTH; 2014 Feb: https://www.cadth.ca/sites/default/files/pdf/htis/apr-2014/RC0519%20-%20Next%20Generation%20Sequencing%20Final.pdf
Accessed 2019 Sep 12.

Non-Randomized Studies

No Comparator

Mestek-Boukhibar L, Clement E, Jones WD, et al. Rapid Paediatric Sequencing (RaPS): comprehensive real-life workflow for rapid diagnosis of critically ill children. *J Med Genet*. 2018 Nov;55(11):721-728.
PubMed: PM30049826

Stark Z, Lunke S, Brett GR, et al. Meeting the challenges of implementing rapid genomic testing in acute pediatric care. *Genet Med*. 2018 Dec;20(12):1554-1563.
PubMed:PM29543227

Guidelines and Recommendations

Unclear Methodology

Cancer Research UK. Policy statement: patient access to molecular diagnostics and targeted medicines in England. London (UK): Cancer Research UK; 2018 Sep: https://www.cancerresearchuk.org/sites/default/files/access_to_molecular_diagnostic_tests_and_targeted_medicines_in_england_0.pdf
Accessed 2019 Sep 12.
See: Single Tests, Panel Tests or Whole Genome Sequencing?

Borghesi A, Mencarelli MA, Memo L, et al. Intersociety policy statement on the use of whole-exome sequencing in the critically ill newborn infant. *Ital J Pediatr*. 2017 Nov 3;43(1):100.
PubMed: PM29100554