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# CLINICAL DIAGNOSTIC WHOLE EXOME SEQUENCING FOR INFANTS IN INTENSIVE CARE SETTINGS: OUTCOMES ANALYSIS AND ECONOMIC EVALUATION

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2019

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CLINICAL DIAGNOSTIC WHOLE EXOME SEQUENCING FOR INFANTS  
IN INTENSIVE CARE SETTINGS: OUTCOMES ANALYSIS  
AND ECONOMIC EVALUATION

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Whole exome sequencing (ES) is an extensive form of genetic testing and increasingly used as a diagnostic tool. Clinical uptake of genome-scale sequencing occurred without clear guidelines for application or robust information regarding potential impact on patient health outcomes or cost of care. For infants in intensive care with suspected genetic conditions, ES can be especially powerful to identify a specific diagnosis and inform crucial decisions about medical care. However, little is known about the cost-effectiveness of ES compared to other diagnostic strategies. This project first assessed the literature on pediatric clinical ES. Then, using electronic medical record, diagnostic laboratory, and hospital cost data, we analyzed and compared outcomes and costs of care for patients with suspected genetic etiologies admitted to intensive care within the first year of life in two patient cohorts: those who had ES (ES, n=368) and did not have ES (No-ES, n=368) as part of a diagnostic workup at a large children's hospital. Molecular diagnostic yield (25.8% No-ES, 27.7% ES;  $p=0.56$ ) and 1-year survival (84.8% No-ES, 80.2% ES;  $p=0.10$ ) were similar between cohorts, while ES patients had higher total cost, diagnostic

investigation cost, and genetic test cost during the index admission and for the year after the date of first inpatient genetics consultation (all  $p < 0.01$ ). ES demonstrated important diagnostic utility for patients with monogenic disease, yet other genetic tests, especially chromosomal microarray, remain important given the burden of chromosomal abnormalities in this population. As clinically applied over the first 5 years, ES does not appear to be a cost-effective diagnostic tool for the broad population of newborns and infants with suspected genetic disease compared to standard diagnostic tests such as chromosomal microarray analysis and panel/single gene testing. Further work is needed to develop outcome measures to capture utility of ES results – both diagnostic and non-diagnostic – for clinicians, patients, and patients' families, and to specify clinical guidelines for appropriate ES application.

## TABLE OF CONTENTS

List of Tables .....	ix
List of Figures .....	xi
Introduction.....	12
Study Aims.....	13
Background .....	17
Conceptual Framework.....	19
Literature Review .....	22
Whole Exome Sequencing in Clinical Practice.....	22
Methodology and Measurement Challenges in Economic Evaluations of Clinical Genomic Sequencing.....	26
Lack of Robust Economic Evidence .....	34
Public Health Significance .....	37
Methods.....	39
Study Design.....	40
Aim 1 .....	40
Aims 2 and 3.....	42
Study Setting.....	43
Aim 1 .....	43
Aims 2 and 3.....	43
Study Subjects .....	44
Aim 1 .....	44
Aims 2 and 3.....	44
Study Power.....	48
Aim 1 .....	48
Aims 2 and 3.....	48
Data Collection.....	50
Aim 1 .....	50
Aims 2 and 3.....	56
Data Analysis .....	84
Aim 1 .....	84
Aim 2 .....	85
Aim 3 .....	87
Main Limitations .....	90



Human Subjects Research: Ethical Considerations .....	92
Journal Articles .....	94
Clinical Application of Genome and Exome Sequencing as a Diagnostic Tool for Pediatric Patients: a Scoping Review of the Literature .....	94
Published in <i>Genetics in Medicine</i> .....	94
Exome Sequencing for Critically Ill Infants Compared to Standard Diagnostics: A Retrospective Analysis of Clinically Matched Cohorts.....	188
Target Journal: <i>Genetics in Medicine</i> .....	188
Cost-Effectiveness Analysis of Clinical Exome Sequencing Compared to Standard Diagnostics for Critically Ill Infants.....	241
Target Journal: <i>Genetics in Medicine</i> .....	241
Conclusion.....	287
References .....	288

## LIST OF TABLES

Table 1. Cost-Effectiveness Analysis Framework – A Checklist of Necessary Components.....	22
Table 2. Measurement Matrix for Aim 1 .....	51
Table 3. Measurement Matrix for Aim 2 .....	58
Table 4. Measurement Matrix for Aim 3 .....	76
Table 1. Summary of Large Sample Studies.....	117
Table 2. Summary of Findings in Economic Evaluation Articles .....	122
Table S1. Neurologic Phenotype Diagnostic Yield.....	124
Table S2. Genes and Associated Diagnoses Reported in Case Studies.....	125
Table S3. Economic Impact Calculations from Large Sample Studies .....	133
Table 1. Patient Characteristics .....	212
Table 2. Index Admission Characteristics .....	214
Table 3. Outcomes .....	217
Table 4. Molecular Diagnosis .....	218
Table 5. Cox proportional hazards regression model .....	221
Table 1. Patient Characteristics .....	264
Table 2. Costs by cohort <sup>a</sup> .....	266

Table 3. Costs, Patients admitted in 2016 and 2017 <sup>a</sup> .....	268
Table 4. Costs by ES recommended but not performed <sup>a</sup> .....	271
Table 5. Cost drivers, index admission <sup>a</sup> .....	273
Table S1. Cost of Index Admission Regression Model .....	277
Table S2. Costs by 28-day survival.....	279
Table S3. Costs, NICU patients only <sup>a</sup> .....	281
Table S4. Costs by form of ES <sup>a</sup> .....	283
Table S5. Costs by diagnosis <sup>a</sup> .....	285

## LIST OF FIGURES

Figure 1. Translation of genomic research to clinical and public health applications .....	21
Figure 2. Study flow diagram.....	46
Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection .....	116
Figure 1. Study flow diagram.....	211
Figure 2. Index admission length of stay (days) for patients who did and did not have ES.....	216
Figure 3. Survival by ES cohort.....	220
Figure 4. Survival by hospital unit, all patients .....	223
Figure 5. Survival by diagnosis category and cohort .....	224
Figure S1. Uptake by exome sequencing test form .....	227
Figure S2. Consults and diagnostic yield by attending geneticist .....	228
Figure S3. Form of exome sequencing orders by attending geneticist .....	229
Figure 1. Total cost of index admission by cohort.....	269
Figure 2. Total cost of index year by cohort.....	270
Figure 3. Mean index admission total cost by cost category and cohort.....	275

## INTRODUCTION

Completion of the Human Genome Project in 2003 marked a major accomplishment in the field of biological research. It fostered an ambitious goal within the scientific and medical community to translate genetic knowledge from the research setting to the clinical setting and use an individual patient's genetic makeup to guide medical decision-making.<sup>1</sup> Rapid reductions in the cost of sequencing, along with identification of clinically relevant gene variants through accumulation of sequence information, have made genome-scale sequencing increasingly relevant for patient care. In clinical practice, whole exome sequencing (ES) has been successfully applied as a diagnostic tool.<sup>2,3</sup>

ES is a powerful test because it can identify a molecular-level diagnosis based on a broad picture of a patient's most clinically relevant genetic sequence information. Using next-generation sequencing techniques, ES simultaneously analyzes the deoxyribonucleic acid (DNA) sequence of the exons in each gene, collectively referred to as the exome. The exome is the subset of the genome that codes for protein products and is most well-understood.<sup>4</sup> ES can potentially replace a wide range of other diagnostic tests and may both reduce the time required to establish a diagnosis and increase the probability that a specific diagnosis is made in a particular patient.

ES is especially useful for individuals with rare diseases or diseases that are difficult or impossible to diagnose with other diagnostic modalities.<sup>5</sup> Care providers can order ES from a clinical genetic laboratory in three different forms: sequencing of the patient only (proband ES), sequencing of the patient and both biological

parents (trio ES) and sequencing of the patient and both parents with a reduced turnaround time (critical trio ES).

While clinical uptake of genomic sequencing is increasing, there is very little evidence on the impact of ES on patient health outcomes or the cost-effectiveness of using ES as a diagnostic test compared to other diagnostic strategies. Moreover, specific and measurable patient outcomes following clinical genomic sequencing (cGS) have not yet been systematically explored or defined. Measurement and analysis of the effectiveness of ES to achieve relevant and specific health outcomes can provide important evidence of clinical utility. Moreover, effectiveness data is a necessary precursory step to economic evaluation. Generation of an evidence base to help understand where and how ES fits into medical care is essential to guide practical and efficient technology translation from the laboratory bench to the patient bedside. In turn, evaluation of bedside use – and the development of appropriate methods for conducting such evaluations – is an increasingly important topic for health services research.

## **Study Aims**

This research addressed the following overarching question: What is the impact of exome sequencing (ES) as a clinical diagnostic tool for critically ill infants with suspected genetic conditions? To investigate this question, three sub-questions were asked: (1) What is currently known about the effectiveness and cost-effectiveness of clinical genomic sequencing (cGS)?; (2) How do relevant and measurable outcomes for infants in intensive care settings compare between

patients who did and did not have ES?; and (3) How does cost of care compare between patients who did and did not have ES, and what is the cost-effectiveness of ES for diagnosis of infants in intensive care compared to usual diagnostic care? Research aims were developed to investigate each sub-question in turn. Motivations for each aim of this research, the aims, and the specific objectives to achieve each aim are listed below.

Motivation 1: A comprehensive review of publications reporting on cGS has not yet been published. cGS use has not been summarized by clinical setting or patient type, and reported outcomes such as diagnostic yield of these tools has not yet been synthesized across studies. Knowledge of how cGS has been applied in practice to date can inform uptake and direct further research.

**Aim 1.** Synthesize the available clinical and economic evidence from the literature on genomic sequencing as a clinical diagnostic tool in pediatric patient populations

Objectives:

- a. Perform a scoping review of published peer-reviewed literature on the use of genome sequencing (GS) and exome sequencing (ES) in clinical practice for pediatric patients (0–18 years of age)
- b. Summarize disease areas, and associated molecular diagnostic yield, in which diagnostic cGS has been used, and identify commonalities and differences in what has been reported

- c. Identify and describe categories of reported sources of clinical utility of cGS and medical management changes following results, as well as how these categories were defined and operationally measured
- d. Summarize the level of evidence in the literature on effectiveness and cost-effectiveness from a health services research perspective

Motivation 2: Identification of an appropriate patient population, comparison population, and outcome measures to use as endpoints in health economic analyses has been identified as a major hurdle for value assessments of cGS. A robust, comparative analysis of the effectiveness of ES to achieve specific outcomes is lacking. Construction of an appropriate cohort of comparison group patients and determination of important and feasibly measurable outcomes are crucial elements of study design to assess the value of cGS.

**Aim 2.** Identify and describe a population of undiagnosed infants with suspected genetic etiology in intensive care settings, define and measure relevant clinical outcomes, and compare outcomes for patients who did and did not have ES.

Objectives:

- a. Provide an overview of practical challenges associated with outcome measurement in cGS
- b. Describe methods for identification of the patient population and procedures used for matching patients who had ES to patients who did not have ES based on clinically relevant features



- c. Provide descriptive statistics on demographics and clinical features of patients included in the study sample
- d. Identify relevant outcomes, conduct retrospective electronic medical record review to compile data on candidate outcome measures, and describe how outcomes were measured
- e. Perform statistical analyses to compare outcomes between cohorts of patients who did and did not have ES and other relevant subgroups

Motivation 3: There is very little evidence on the cost-effectiveness of clinical diagnostic ES for infants in intensive care settings. Evidence from economic evaluation is needed to inform the clinical use of the test and payers' medical coverage policy.

**Aim 3.** Compare cost of care for patients who did and did not have ES as part of a diagnostic workup and calculate incremental cost-effectiveness of ES, compared to a diagnostic pathway that does not include ES, for critically ill infants less than 1 year of life with a suspected genetic etiology at Texas Children's Hospital

Objectives:

- a. Analyze costs for cohorts of patients who did and did not have ES over the index admission and one year following the initial genetics consultation for categories of total cost, hospital billing code, diagnostic pathway, and genetic tests.

- b. Compare costs and outcomes over the time horizon of the index admission and one year following the initial genetics consultation using the hospital perspective. Primary outcomes by which to measure effectiveness include molecular diagnostic yield and survival.
- c. Calculate incremental cost-effectiveness ratios (ICERs) for ES for relevant outcome measures

## **BACKGROUND**

Whole exome sequencing (ES) demonstrated proof of concept to diagnose a patient in the research setting in 2009.<sup>6</sup> ES became commercially available as a clinical test in 2011, meaning that a clinician could order it from a diagnostic laboratory. It generally utilizes a blood sample to generate the deoxyribonucleic acid (DNA) sequence of the patient's exome – a word used to collectively refer to the approximately 180,000 exons, the portion of the genome that encodes instructions for making protein products that is made up of about 30 million base pairs, or 1-2% of the total genome sequence.<sup>6</sup>

ES was first available as a test for the proband (i.e., the patient for whom the genetic investigation is being performed). Two additional forms of ES, trio ES introduced in October 2014 and critical trio ES introduced in April 2015, require blood or saliva samples from both biological parents of the proband. Parental sequence information increases the ability of medical geneticists to differentiate between inherited sequence variants and *de novo* mutations in the child.<sup>7</sup> For example, a sequence variant detected in the patient is less likely to be causal of

disease presentation if it was inherited from a healthy parent. The proband's DNA sequence is compared to a reference sequence and the sequence of both parents, and sequence variants are interpreted alongside clinical phenotype, variant databases, and disease gene literature.

Critical trio ES was developed for use in critically ill patients and has a two to three week turnaround time, compared to eight to ten weeks for return of results from proband-only and trio ES. Reduced turnaround time is important when the results of the test are needed to guide urgent medical management decisions. Definitive knowledge of the genetic basis of disease within a short timeframe may be especially influential in the neonatal intensive care unit (NICU) and pediatric intensive care unit (PICU).

Establishment of a molecular diagnosis can inform critical medical decisions that impact health outcomes, and time to diagnosis can impact the availability or effectiveness of clinical intervention.<sup>3,8-10</sup> A correct molecular diagnosis can guide the initiation or discontinuation of therapy, medication, or diet. It can also inform the need for further testing, prognosis, anticipatory care, and whether palliative care initiation or withdrawal of support is appropriate.<sup>3,10</sup> Length of hospital stay may also be directly or indirectly affected by quicker result reporting. Therefore, in high-cost, high-intensity care settings such as the NICU and PICU, turnaround time of a diagnostic test can potentially affect both costs and outcomes.

Quantification of the effect of incorporating ES into a diagnostic pathway is needed to inform both clinical policy and health care payer policy. A diagnostic workup for infants with a suspected genetic etiology may include many diagnostic

modalities (e.g., imaging, metabolite screening, targeted genetic testing), which we refer to as usual diagnostic care. There are no clearly delineated diagnostic pathways for the heterogeneous population of NICU patients. The diagnostic pathway may include only usual diagnostic care or one of the three ES forms. Patient outcomes following alternative strategies for diagnosis and the cost per measure of effect can be calculated and compared to quantify the value of clinical diagnostic ES.

This project provided a practical approach to outcome measurement and incorporated those outcome measures into a full economic evaluation of ES for infants in intensive care settings. It specifically evaluated clinical uptake and application of ES as a diagnostic tool. As such, it did not include analysis of other genomic applications, such as pharmacogenetic testing, tumor genotyping, prenatal genetic testing, or direct-to-consumer genetic tests.

## **Conceptual Framework**

This study can be positioned within conceptual models of technology translation from laboratory science to clinical application, commonly referred to as “bench to bedside.” The Office of Public Health Genomics at the Centers for Disease Control and Prevention was the first to apply a translation research framework to the field of genetic medicine.<sup>11,12</sup> The framework takes a public health perspective and emphasizes the need for multidisciplinary research in genetics. Beyond basic science research, there are four stages of translation research associated with connecting advances in basic science genetics to patient care and ultimately

population health. The feedback loop between stages of translational research is depicted in Figure 1. As characterized by Khoury, Gwinn, Yoon, Dowling, Moore, Bradley <sup>12</sup> the stages of research are as follows:

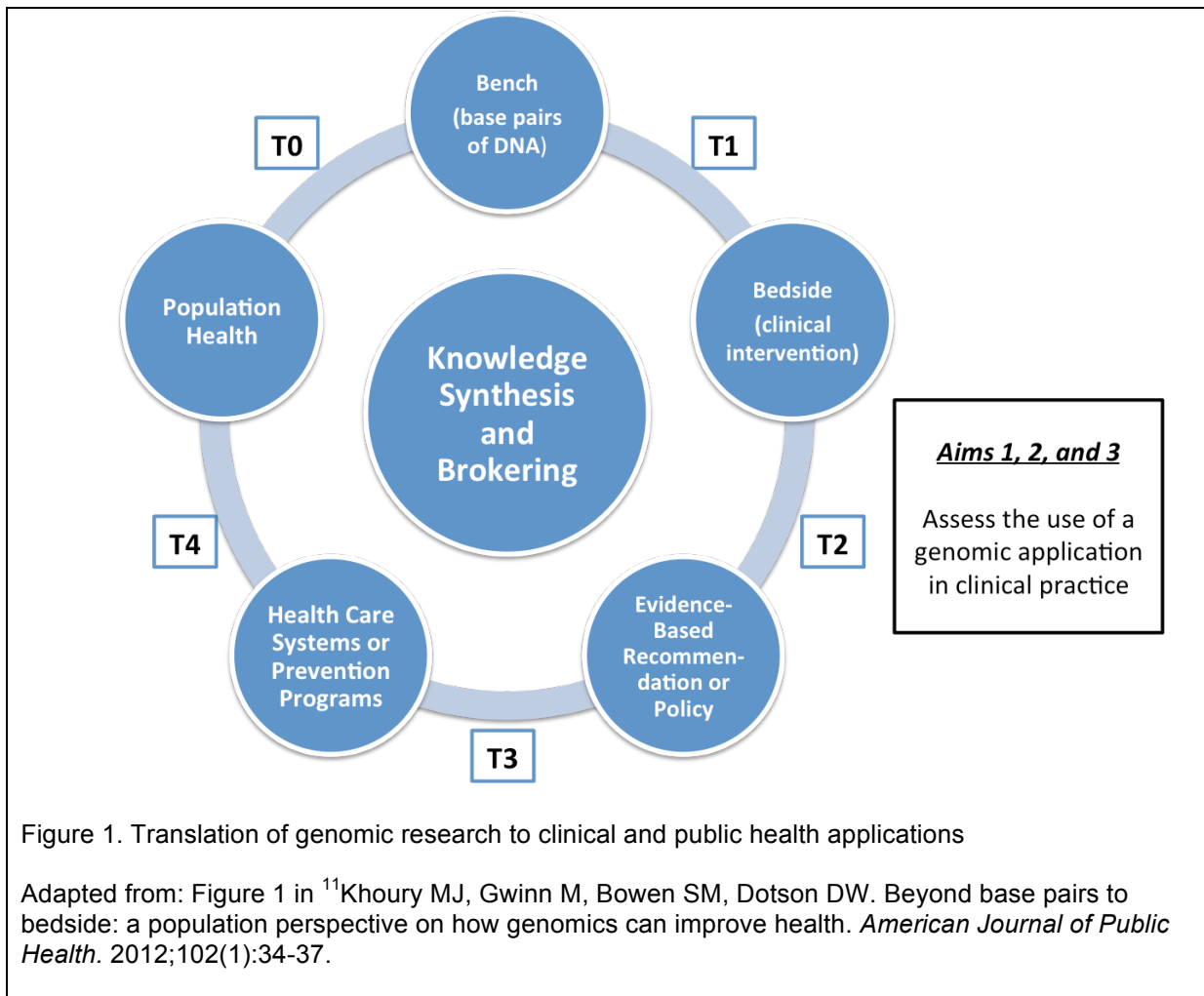
T0 – Basic science research

T1 – Development of basic science research into an application, a product such as a genetic test, for use in the clinical setting

T2 – Assessment of a genomic application in clinical practice and development of evidence-based practice guidelines for its use

T3 – Analysis of the clinical implementation of a genomic application according to evidence-based practice guidelines through delivery, dissemination, and diffusion research

T4 – Evaluation of the population health impact of a genomic application through outcomes research



The crux of T2 stage research is to determine the impact of a genomic application on patient health and the associated value. Results of such analyses are then used to inform development of evidence-based practice guidelines. Thus, economic evaluations used to determine the value of a test or intervention are situated in the continuum of translation of genetic research at the T2 stage.

Full economic evaluations of a health care intervention consider both the costs and health outcomes of two or more strategies.<sup>13</sup> Cost-effectiveness analysis (CEA) is a framework used to compare the cost of alternative courses of action with

associated health outcomes, which are measured in natural units. A CEA can inform decision-makers about the value of a genomic application and provide evidence for consideration in practice guideline development. There are defined criteria for CEAs conducted in the realm of health and medicine, and Table 1 displays a checklist of items that should be considered and reported in a formal CEA.

Table 1. Cost-Effectiveness Analysis Framework – A Checklist of Necessary Components
Well-defined question in answerable form
Comprehensive description of the competing alternatives
Effectiveness of the service established
All important and relevant costs and consequences for each alternative identified
Costs and consequences measured accurately in appropriate physical units
Costs and consequences valued credibly
Costs and consequences adjusted for differential timing
Incremental analysis of costs and consequences of alternatives
Allowance made for uncertainty in the estimates of costs and consequences
Presentation and discussion of study results includes all issues of concern to users
Adapted from Box 3.1, p. 28 of <sup>13</sup>

## Literature Review

### ***Whole Exome Sequencing in Clinical Practice***

Provision of medical care based on a patient's genetic sequence information has been an ambitious goal of the scientific and medical community since the idea of the Human Genome Project was first conceived. The ability to relate basic science

research in the field of genetics to patient care requires development of genomic applications that are useful in the clinical setting. Translation of genomic technologies from the research setting to the clinical setting is a hallmark of the beginning of a new era of clinical care – the era of genomic medicine – exemplified by clinical diagnostic ES.<sup>1</sup> Ultimately, the intent of genomic medicine is to improve the health of individual patients by tailoring therapy to their specific genetic makeup.

A combination of advances in genetic basic science and development of corresponding clinical applications have created the conditions for translation of genomic sequencing into the patient care setting. The rapid decline of technical costs of DNA sequencing, development of faster next-generation sequencing (NGS) technologies, and increased understanding of molecular biology of disease have made genetic sequencing increasingly relevant to medical practice. Unlike whole genome sequencing (WGS) which sequences the more than three billion bases in the entire human genome, only the exome is sequenced in ES. Because ES involves sequencing fewer chemical bases, it also costs less to perform than WGS. The cost to sequence an exome is now near \$1,000 – the benchmark cost at which scientists have long held that the sequencing of every patient is justified.<sup>14</sup> The exome is the protein-coding region of the genome, meaning that it contains information required to make the “material” end products of genetic sequence information. Although the exome only constitutes less than two percent of the entire genome, it is where approximately 85% of disease-causing mutations lie and is the most well-understood portion.<sup>4,15</sup> Therefore, the most clinically meaningful sequence information, with the greatest potential for medical actionability based on findings, is



obtained through ES. At reasonably attainable costs and accompanied by a greater understanding of the genetic basis of disease (due largely to accumulation of sequence information itself as more patients are sequenced, leading to discovery of new disease-causing genes), ES is anticipated to have widespread application to clinical diagnosis in the future.

As a result of the volume of information ES provides, it may be used to diagnose any number of conditions across multiple disease categories. ES has a broad scope as a diagnostic tool because it simultaneously analyzes all of the coding variation in a human genome, even in areas that are not yet well understood clinically. This makes it particularly powerful in the context of rare diseases, and results from ES are a major source of information on rare disease-related gene variation.<sup>16</sup> ES can provide insight that would not be possible using more targeted genetic tests, such as single gene or gene-panel tests that only provide information on specific coding regions. ES conveys information on pathogenic genetic variants that may occur anywhere in the exome, including in genes for which single gene tests do not exist or a clinician may not know to order.<sup>17</sup> It is especially useful for individuals with rare diseases or diseases that are difficult or impossible to diagnose using other diagnostic modalities such as imaging, biopsies, cerebrospinal fluid examination, and electromyography or even other types of genetic testing such as chromosomal microarray and targeted single or gene panel tests.<sup>4</sup>

ES has demonstrated molecular diagnostic proof-of-concept and clinical utility to impact the course of patient care in clinical practice.<sup>2,7,17-27</sup> Most studies are on pediatric populations with undiagnosed disease with suspected genetic etiology,

particularly patients with neurodevelopmental disabilities or some other neurologic phenotype. Obtaining sequence information for parent-child trios often results in a higher diagnostic yield (i.e., larger proportion of patients diagnosed out of all patients sequenced).<sup>2,28</sup> The reported molecular diagnostic yield ranges from approximately 25% to 58%. Results of ES have reportedly influenced changes in drug therapy, surgery decisions, understanding of inheritance pattern, palliative care initiation, and understanding of risk for future pregnancies (i.e., risk for potential future siblings of the proband to also be affected by an inherited disorder).

The potential for ES to impact patient care has received special attention in the neonatal intensive care unit (NICU) setting, where it may be distinctly valuable as a diagnostic tool. ES is helpful in diagnosing congenital malformations, especially for newborns in which the clinical presentation is atypical or the phenotype is not yet fully developed, which hinders the ability of clinicians to make a clinical diagnosis.<sup>10,29</sup> Although ES may reduce the length and cost of the diagnostic odyssey in patient populations for which other forms of testing do not yield a diagnosis, the time required to generate the data and format a clinical report can limit the utility of non-rapid ES diagnostic test for critically ill patients. Turnaround time can be crucial in the NICU and PICU, where time to diagnosis and establishment of a molecular diagnosis can inform critical medical decisions.

In addition to ES, other forms of next-generation sequencing are being applied in the NICU, including WGS and rapid, expanded panel sequencing.<sup>9,30-32</sup> WGS has demonstrated proof-of-concept for diagnosis in the NICU.<sup>10,33</sup> Research-based rapid trio WGS in the level 4 NICU and PICU at Children's Mercy Hospital

provided diagnosis for 20 of 35 (57%) acutely ill infants less than four months of age with suspected genetic disease. Standard genetic testing failed to identify 18 of the 20 diagnoses. Studies of this patient population suggest that WGS can increase 28 day mortality while decreasing one year mortality, most likely through its impact on the decision of whether to withdraw life support.<sup>10,23</sup> Of the molecular diagnoses, 20% led to a positive clinical impact and 30% led to the initiation of palliative care.<sup>23</sup>

### ***Methodology and Measurement Challenges in Economic Evaluations of Clinical Genomic Sequencing***

Very little is known about the cost-effectiveness of cGS. The need for robust evidence from economic evaluations has been widely recognized, and implementing institutions often identify the need for cost-effectiveness information when describing their initial applications of clinical ES.<sup>17,19,20,22,34</sup> Further study and more robust evidence are considered requisite by some institutions before cGS is incorporated into routine clinical care plans.<sup>23</sup> Most peer-reviewed literature on applications of ES is written from a clinical genetics perspective. These studies usually have a small sample size (typically 40 or fewer) of heterogeneous patients from a single institution. Molecular diagnostic yield is a commonly reported outcome measure, which is usually presented as a raw calculation and does not account for the influence of a multitude of factors by which it may be affected.<sup>35</sup> If any change in clinical management is discussed, it is typically reported in the style of a case report or case series. Most studies are retrospective and do not include a comparison

group. However, one recent prospective analysis of diagnostic ES suggests that it is cost-effective, and potentially even cost-saving as a first-line test.<sup>36</sup>

Outcomes research and economic evaluations of cGS are sparse because they face a host of methodological issues. There is no consensus among health economists as to what the methodological approach should be, and there is a question as to whether traditional economic evaluation approaches are applicable to genetic services or whether new methodology must be developed.<sup>37,38</sup> In a review of published discussions of methodological challenges surrounding the economic evaluation of genomic services, Buchanan, Wordsworth, and Schuh<sup>39</sup> summarized the specific challenges as development of methods to incorporate effectiveness data, costing of sequencing platforms, and measurement of health outcomes. The Institute of Medicine's Roundtable on Translating Genomic-Based Research for Health identified four categories of challenges that surround the economic evaluation of genomic medicine: (1) incongruent disciplinary perspectives and language barriers between economists and geneticists; (2) insufficient outcomes evidence and lack of standardized thresholds for evidence and willingness to pay; (3) dynamic nature of genome data and inability of traditional economic assessments to keep pace; (4) need for development of methods to incorporate personal utility.<sup>38</sup>

Costing of genomic sequencing procedures presents a unique set of challenges. The appropriate unit of analysis for costs is the testing service, which includes the cost of medical geneticists' effort involved in interpretation of results and pre- and post-test genetic counseling, plus lab costs such as chemical reagents required for sequencing, bioinformatics pipeline development, data storage, and

quality assurance/quality control practices.<sup>40-42</sup> Moreover, the cost of ES and placement in the diagnostic pathway, which is determined by provider behavior, impacts the cost per diagnosis. Parameter values change quickly as a result of relatively rapid changes in basic science in genomics and clinical practice in the field of genomics.<sup>40</sup>

Outcome measurement has been identified as a major challenge in evaluating clinical genetic services and providing evidence of medical benefit to payers.<sup>12,38,43-46</sup> Because genomic testing has created a new paradigm for clinical diagnosis, it does not conform to the established method of comparing a new test to the existing gold standard.<sup>47</sup> In a systematic literature review of 342 health technology assessments and economic evaluations of genetic testing technologies, the majority (62%) of reviewed studies were CEAs, and 75% of the CEAs used intermediate outcome measures such as the number of cases detected.<sup>48</sup> An intermediate measure is not traditionally regarded as an appropriate endpoint in a CEA. Ideally, such analyses of alternative interventions compare final health consequences, measured in natural units such as life-years gained.<sup>13</sup> In the case of genetic testing where information is the immediate outcome, arriving at a final health outcome measure is methodologically challenging and may not be pragmatic because it requires systematically measuring not only immediate changes in medical management but also impacts on health over a long term.<sup>49</sup> However, establishment of a molecular diagnosis may be regarded as an appropriate endpoint because it can have some value in and of itself, apart from its potential effects on care rendered. Moreover, for conditions without available treatment, the value of a

diagnostic test may be determined by its ability to correctly identify the underlying cause of disease.<sup>50</sup> The objectives of decision-makers and stakeholders for whom the analysis is performed determine whether a molecular diagnosis is an acceptable outcome measure.<sup>13</sup> The clinical utility for any specific molecular diagnosis is partially determined by current availability of treatment options, and thus may change over time as new therapeutics become available.

Measurement of effectiveness and the concept of utility in the realm of genetic medicine have been widely discussed. Two main perspectives on the concept of clinical utility have emerged. Narrowly defined, clinical utility is the impact of a test on medical management of the patient. A more broad definition of clinical utility encompasses other aspects of genetic tests for the patient and family, and the relevant factors depend upon which stakeholders' perspective the analysis is conducted from.

Under the first view, clinical utility of a test or service is determined strictly by its impact on health outcomes of the patient via guidance of a change in medical management. In the context of ES, results from the test may inform clinical decisions that have direct implications for the patient; multiple scenarios have been documented in practice.<sup>20-22,26,51,52</sup> ES may lead the provider to initiate a change in management or treatment plan, such as initiation or discontinuation of medications, therapy or other diagnostic tests. Results may also guide anticipatory or palliative care. Additionally, knowledge of sequence information may avert a potentially harmful misdiagnosis. Establishment of a molecular diagnosis enables providers to make a more accurate prognosis. For patients with certain molecular diagnoses,

genotype information may make them eligible for clinical trials of a relevant therapeutic.<sup>21,25</sup>

Under the second view, clinical utility depends upon the stakeholder's perspective from which the analysis is conducted. For example, third-party payers are interested in test results providing an accurate, timely diagnosis that impacts clinical management or ends the “diagnostic odyssey” – the prolonged search for the cause of illness which often involves numerous forms of invasive testing, multiple specialist visits, and can last for years. Establishment of a molecular diagnosis via ES may lessen the duration, expense, and invasiveness of the diagnostic odyssey.<sup>20,25,52-54</sup> From a healthcare payer perspective, the ability of ES to shorten the diagnostic odyssey is key because paying for one test early in the diagnostic pathway might potentially avoid charges for multiple unsuccessful tests and consultations later on, and it can also replace multiple specific tests. Thus, a receipt of a diagnosis is a relevant endpoint.

Genetic sequence information obtained through ES can also have impacts that extend beyond the patient to other members of the family. ES results can inform reproductive risk assessment and reproductive options for the proband, but they can also aid in family planning decisions for the parents of the proband. Results may also guide disease screening for family members, especially if the genetic information leads to a change in the presumed inheritance pattern of the condition that implicates disease risk in a family member.<sup>21</sup> Families might value information for career and residential planning.<sup>55</sup> Receipt of a diagnosis may have value in and of itself, even if the diagnosis does not affect health outcomes directly.<sup>46</sup> In addition

to providing information useful for planning in various aspects of familial life and the possibility to engage in disease support groups, ending the diagnostic odyssey can relieve the family of a significant psychological and financial burden that comes with continued searching for the cause of a child's illness.<sup>56,57</sup>

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group, established by the Office of Public Health Genomics of the Centers for Disease Control and Prevention, has stated that “hard” clinical outcomes conventionally used in evaluation of diagnostic tests should be considered alongside “soft” (i.e., behavioral) outcomes when evaluating genomic technologies.<sup>46</sup> This is based on the notion that genomic sequencing involves many more aspects of the patient's life – and the lives of family members – than just their health state. Capturing such broad effects is not possible with outcome measures commonly used in economic evaluations, such as quality-adjusted life years (QALYs), which do not incorporate non-health related outcomes. Thus, most economic evaluations of genetic testing have been CEAs rather than cost-utility analyses because appropriate metrics for utility in the genomic arena have not yet been developed.<sup>37</sup> In addition, measurement of psychological effects requires longitudinal assessment, which is difficult in practice. Such psychological effects may be important, however, especially when assessing the value of newborn genetic sequencing. The BabySeq project, a randomized controlled trial of newborn genomic sequencing funded by the National Institutes of Health, is evaluating psychological impacts on the family as well as clinical and economic outcomes.<sup>58,59</sup>



The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (EWG), an independent panel supported by the CDC's National Office of Public Health Genomics, specified the components of evaluation of a genetic test. Known as the ACCE criteria, the factors for consideration are analytic validity, clinical validity, clinical utility, and ethical social, and legal implications.<sup>60</sup> Analytic validity is defined as the ability of the test to accurately and reliably measure the genotype of interest in the lab, which includes analytic sensitivity, specificity, and reliability. Clinical validity is defined as the ability of the test to accurately and reliably predict the clinically defined disorder or phenotype of interest, which includes clinical sensitivity and specificity (that incorporate analytic sensitivity and specificity), positive and negative predictive values considering characteristics of the population of interest, and the molecular attributes of the genotype. Clinical utility of the test is defined as its effect on measurable clinical outcomes and usefulness in guiding decisions about patient care management compared with current management without genetic testing. When the EWG employs this criteria to assess outcomes of genetic tests, the outcomes of interest are tailored to each clinical scenario for which the use of the test is being evaluated.<sup>46</sup>

The EGAPP definition of clinical utility as an impact on medical management emphasizes that a critical piece of information required to assess the value of a genetic test is how it influences the medical decision-making process. The role of physician behavior in patient health outcomes is also necessary to consider. Once the results of a diagnostic test are returned, a change in clinical practice (e.g., initiation of a treatment change, ordering of other tests, withdrawal of support)

precedes a change in patient outcomes (e.g., survival, quality of life, disease progression, response to therapy). As formulated by Peabody and colleagues,<sup>61</sup> physician behavior lies along the causal pathway to patient outcomes.

Due to many of these methodological challenges, there is a lack of robust economic evaluations of cGS, and the cost-effectiveness of different forms of ES compared to other diagnostic strategies has not been performed. A systematic review of health economic evidence on genome sequencing found that the few studies published through May 2013 are merely cost calculations, and that poor methodology limits the accuracy of the findings.<sup>62</sup> Most studies aim to describe genetic findings; they do not formally assess costs. Calculations of per-sample costs are usually incomplete because they do not include the substantial indirect costs associated with genetic testing such as genetic counseling, clinical geneticist consultations, bioinformatics pipeline and protocol development, variant validation tests, and overhead.<sup>42</sup>

Such gaps in evidence highlight the need for multidisciplinary research in genomic medicine. From a public health perspective, in order to quantify the full value of genomic sequencing procedures, assessments must be performed at each stage of the translation process. A more robust evidence base is necessary to ensure appropriate applications of genomic medicine that ultimately improve population health. Currently, T2 stage evaluations of promising applications such as diagnostics are particularly deficient and needed.<sup>11</sup> Absence of an evidence base hinders the development of practice guidelines and appropriate uptake of genomic services. As of 2007, it was estimated that three percent or less of research

published in the area of genomics focuses on evaluation of a genomic application after it is in use (T2-T4 research), and T2 research in human genetics has been called “inconsistent and nonsystematic.”<sup>63,64</sup> Systematically assessing data from individual medical centers that have implemented particular genetic applications in isolation can help develop a foundation for best practices.<sup>1</sup> Such assessments can guide efficient uptake and use of services in other institutions.

Although provider behavior is an essential element of implementation, research on how ES results inform medical decision making has received even less attention than costs. In intensive care settings, decisions with especially high stakes may be made on the basis of ES results (i.e., the decision to withdraw support). Care decisions involve multiple stakeholders. Clinical judgment of providers plays a crucial role, but respect for autonomy requires that parents of critically ill infants be informed of test results and allowed to participate.<sup>65</sup> More attention has been devoted to parental decision support tools to help parents decide whether to have their child’s exome sequenced than to the parent’s involvement in medical decision making once results are received.<sup>66</sup> Survey data suggest that parents are interested in the possibility of whole genome sequencing of newborns (integrated with current newborn screening program).<sup>67</sup> However, this might not apply in the context of an intensive care setting where medical decisions are imminent.

### ***Lack of Robust Economic Evidence***

There are several limitations of work to date on evaluations of genomic sequencing procedures. There is virtually no evidence from large-scale randomized

controlled clinical trials. Nearly all studies are retrospective, apart from one recent prospective parallel study of ES on 40 critically ill infants.<sup>26</sup> Even well-designed retrospective studies to date are based on a small sample size; for example, 35 families met inclusion criteria for the NICU study by Willig and colleagues.<sup>23</sup> A randomized controlled trial of rapid WGS in NICUs is in the design stages, and a cost-effectiveness analysis is planned to take place alongside the trial.<sup>68</sup> However, in order to harness information from cGS that has already been performed and because randomized controlled trials will not always be appropriate or pragmatic, approaches for comparative effectiveness research that utilizes existing records should be developed.<sup>64</sup>

Patient heterogeneity within the sample is a substantial analytic hurdle for both prospective and retrospective study designs. Most studies to date include all patients (or families) who underwent genetic testing in the analysis, leading to a diverse array of molecular causes of disease within patients studied. Cost-effectiveness may be different for different clinical scenarios. The clinical settings or patient groups in which genomic diagnostics have the highest clinical utility are not yet known. Determination of these factors has been designated as an important research focus.<sup>64</sup> Knowledge of particular situations in which ES is cost-effective can inform efficient clinical use and payer policy.

Heterogeneity also exists in provider behavior. While little is known about uptake of cGS among physicians, there is some evidence of a “silo” approach, which describes implementation being driven by a particular individual or department within an institution.<sup>1,27</sup> The decision of a physician to order ES may be influenced by

availability, departmental standards, peers, or individual research interests. Lack of established practice guidelines on cGS makes choice of comparators difficult because each provider may order sequencing based on different criteria or at a different point in the diagnostic pathway, and cost-effectiveness is always dependent upon the clinical context. Most CEAs of genetic testing use decision analytic modelling to incorporate available evidence. However, the appropriateness of parameter values used to perform the analysis has been questioned.<sup>48,69</sup> Epidemiologic studies from which parameters are taken is often not properly considered, and uncertainty in the parameter values is often not properly accounted for through sensitivity analysis.

Absence of robust economic evidence has created a barrier to clinical ES in some circumstances. Although costs of next generation sequencing procedures have substantially decreased, they remain unaffordable for many patients. Out-of-pocket costs are one of the largest practical barriers to translation of ES technology from bench to bedside. Indeed, insurance coverage has been referred to as the “fourth hurdle” in technology translation from basic science to clinical use.<sup>70</sup> ES charges can range between \$4,500 and \$9,000. Even in cases of partial coverage, the mean out-of-pocket expense for patients involved in one study was \$1,082.13 (range \$279—\$2,500), and some patients who were referred for ES and approved by clinical review boards decided not to undergo testing after coverage denial.<sup>34</sup> Clinical geneticists have expressed concern about insurance coverage and the lengthy insurance approval process.<sup>71</sup> Burdensome administrative requirements or denial of coverage is a potential barrier to patient access to cGSs.

Private payers have been reluctant to cover sequencing procedures largely because they regard the technology as unproven. Insurers require more data on clinical utility and cost-effectiveness before they will consider genomic sequencing as anything other than “experimental” or “investigational.” Lack of robust evidence on clinical utility has been cited as a major barrier to convincing payers to cover the service.<sup>1,61</sup> Little or no evidence exists in the form typically required for coverage of a new technology or service – namely proof of analytic validity, clinical validity, and clinical utility, formal health technology assessments, and practice guidelines – for all of the reasons related to practical and methodological challenges discussed above.<sup>72,73</sup> From a payer perspective, Sabatini and colleagues demonstrated that whether use of ES was cost-saving or cost-increasing depended upon clinical features of the patient population, the cost of the test, and where it was incorporated in the diagnostic pathway.<sup>74</sup> Development of practice guidelines is thus an important component of an evidence-based use and coverage feedback loop.

### **Public Health Significance**

Ability to more quickly and precisely diagnose genetic disorders and provide appropriate diagnosis-based care can substantially impact public health.

Collectively, rare and genetic-based diseases are associated with substantial disease burden and societal cost.<sup>75,76</sup> Congenital malformations and chromosomal abnormalities are the leading cause of death for children under one year of age in the United States.<sup>77</sup> Genetic disease diagnoses account disproportionately for neonatal and pediatric hospital admissions and are consistently associated with

higher total charges and longer length of inpatient stay.<sup>78-80</sup> In 2012, admissions associated with a suspected genetic disease diagnosis accounted for an estimated \$14–\$57 billion, representing 11–46% of total charges for all inpatient admissions of patients 0 – 20 years of age.<sup>76</sup> Based on national estimates, up to 14% of inpatient pediatric admissions are associated with genetic disease, at a mean cost of up to \$77,000 higher for neonatal admissions and up to \$17,000 for pediatric admissions, compared to patients who did not have a genetic disease diagnosis.<sup>76</sup>

Tools of precision medicine relate to public health through application of diagnostics such as clinical genomic sequencing (cGS) to subpopulations who stand to benefit the most from them, such as critically ill newborns and infants.<sup>81</sup> Use of genomic technology and data-driven approaches to provide care for a defined group of patients has been conceptualized as a “precision public health” approach.<sup>82</sup> cGS can potentially lead to more efficient diagnosis and effective treatment, with the anticipated impact of reduced diagnostic odyssey extent, improved outcomes, and associated cost-savings for the health care system.

This project contributes to the field by synthesizing the evidence to date on diagnostic cGS application for pediatric populations and associated outcomes and costs. Further, this study provided a practical example of methods for defining and matching cohorts of patients to perform comparative analysis. It resulted in creation of a unique dataset on patients receiving care at a large children’s hospital over a period of more than 5 years that merged information from multiple sources, including extensive electronic medical record review. This study quantified the relative effectiveness and cost-effectiveness of exome sequencing (ES) as a diagnostic

compared to standard diagnostic care for infants in intensive care. It provided insight into the relative performance of various genetic diagnostic tools in a broadly defined patient population with suspected genetic disease. Results can inform clinical policy and help guide evidence-based precision medicine in neonatology and pediatrics. In turn, findings may impact patient access to cGS via health care payer medical policy development, institutional uptake, and public research investment in translational genomics.

## **METHODS**

The goal of this research was to generate evidence regarding the use of ES as a clinical diagnostic tool. It examined and synthesized published academic literature to provide a scoping overview of available evidence on clinical use of ES. It also provided a practical example of electronic medical record review for outcomes research on a genomic application. Outcomes were described and compared based on use of ES in the diagnostic pathway, clinical setting, and relevant patient characteristics. Cost-effectiveness of diagnostic strategies – no ES versus ES testing (proband-only, trio, and critical trio) – for infants in an intensive care setting was estimated by comparing costs of diagnostic pathways and relevant outcome measures.

Data came from Texas Children's Hospital (TCH) electronic medical records (EMR) and administrative databases and Baylor Miraca Genetics Laboratories (BG) ES result reports. All ES orders from TCH were performed at BG. Each research aim was designed to contribute information regarding the health impact and value of



genomic sequencing as a clinical diagnostic tool for infants in an intensive care setting. This Methods section provides details on the methods used to achieve each aim.

## **Study Design**

### ***Aim 1***

A scoping review of published peer-reviewed literature was performed. Scoping reviews are intended to provide an overview of the nature of literature on a topic via structured searches and identify gaps in knowledge. Fewer restrictions for inclusion are placed on patient population, intervention, outcome, and study design than in systematic reviews. The review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines,<sup>83</sup> adapted for use in a scoping review as appropriate. CGS is defined to include WGS and ES. Sequencing may have been performed for the proband (i.e., patient) only or alongside parents or other family members (duo or trio), in a non-rapid or a rapid manner with reduced turnaround time. Sequencing was considered clinical rather than research for the purpose of this review if the report's stated goal was to make a diagnosis or otherwise impact medical management of the patient(s). In contrast, if the objective was gene discovery or disease mechanism elucidation, the sequencing was considered research.

A search strategy was designed with the assistance of a librarian from the Texas Medical Center library. PubMed, Embase, and Cochrane Library were searched. The PubMed search included the following Medical Subject Headings

(MeSH) terms: Genome; Exome; Sequence Analysis, DNA; Adolescent; Child; Infant; Diagnostic Techniques and Procedures; Clinical Decision-Making; Diagnosis, Differential. Items identified through database searches were imported into the web application Rayyan (Doha, Qatar) for title and abstract screening.<sup>84</sup> Title and abstract of each record were screened by two independent reviewers, and conflicts were resolved through consensus. Citations selected for full-text review were imported into EndNote (Clarivate Analytics, Boston, Massachusetts), and full-text articles were obtained. A full-text review form was completed for each article to determine whether inclusion/exclusion criteria were met. One author reviewed each full-text article, and a second reviewer reviewed a randomly selected 10% of the full-text articles.

Articles that met the following pre-determined criteria were included: (1) peer-reviewed original research article; (2) published between January 2009 and June 2017 (with an updated search performed in November 2017); (3) proband (if a case report) or the majority of probands (if more than 5 probands in study) less than 19 years of age at the time of sequencing; (4) described/evaluated the clinical application of a CGS for diagnostic purposes. Studies of patients who had a clinical diagnosis of a condition with known genetic heterogeneity, and thereby not determined to have a “specific” diagnosis, were included. Studies of patients enrolled in a research protocol performing CGS for a clinical purpose were included regardless of how costs of sequencing were covered, as the aim of sequencing was considered more important than the funding arrangement. No restrictions were placed on study design; clinical reports (individual cases and case series),

intervention studies (any methodology), and economic evaluations (any methodology) were included.

Publications with a primary aim of genetic research were excluded as were publications on population-based screening, tumor genotyping, mitochondrial genome sequencing only (without the nuclear genome), pharmacogenetic testing, disease carrier testing, prenatal genetic testing, and targeted exome sequencing (e.g., “clinical exome” or “Mendeliome”) panels of thousands of genes known to be associated with single-gene disorders. While targeted exomes may be considered more similar to a whole exome than targeted panel, multiple permutations of such tests exist. Because there is inconsistency in covered genes, publications on targeted tests were excluded for comparability of results and feasibility of this review. Reports on patients who were sequenced post-mortem and those that indicated the initiation of sequencing but not results were also excluded.

### ***Aims 2 and 3***

This was a retrospective cohort study in which ES was the exposure factor. Two patient cohorts were defined; patients who had ES as part of a diagnostic workup (ES cohort) were matched based on clinical characteristics and phenotypic presentation to patients who did not (No-ES cohort).

Data on admission characteristics, demographics, phenotypic presentation, clinical outcomes, ES order and result return, and ES uptake by attending clinician was collected through retrospective EMR review. Establishment of a molecular diagnosis and survival were the primary outcomes of interest. A retrospective

approach was optimal in order to utilize information on the large number of patients who had already had ES, ensure follow-up time of at least one year over which to measure outcomes, and enable comparison of multiple outcomes.

## **Study Setting**

### ***Aim 1***

This study was performed in the Department of Molecular and Human Genetics at Baylor College of Medicine.

### ***Aims 2 and 3***

This study was performed in the Department of Molecular and Human Genetics at Baylor College of Medicine. Clinical care was provided at Texas Children's Hospital (TCH) and genomic sequencing was performed at Baylor Miraca Genetics Laboratories (BG), both located in Houston, TX. TCH is a not-for-profit health care organization with large acute critical care capacity. Each year, more than 6,000 children are admitted to TCH ICUs, which have 116 intensive care beds. The TCH NICU is certified level IV, the highest level of care available for premature and critically ill newborns. BG is one of the foremost genetic testing laboratories in the US and was the first to describe ES's application to clinical diagnosis.<sup>2,19</sup> It is accredited by the College of American Pathologists (CAP) certified under the US Centers for Disease Control and Prevention Clinical Laboratory Improvement Amendments of 1988 (CLIA) to return clinical-grade results. BG offers proband-only, trio ES, and critical trio ES, all of which must be ordered by a clinical care provider.

Each ES report is reviewed by a laboratory director and signed out by a molecular geneticist.

## **Study Subjects**

### ***Aim 1***

Not Applicable.

### ***Aims 2 and 3***

The target population for this study is undiagnosed newborns infants in intensive care with suspected genetic etiology. Patients who had a consultation from the Genetics service during an intensive care unit inpatient stay at TCH within the first year of life were included. The study timeframe was December 1, 2011 (when the first ES order was placed for an infant at TCH, very soon after BG offered it as a clinical test) through June 30, 2017. A Genetics consultation documented in the medical record indicated a suspected genetic etiology in the patient. Figure 2 illustrates the flow of patient selection.

Included patients met the following criteria:

- TCH intensive care inpatient admission within the first year of life
- Inpatient genetics consultation documented in the medical record
- Inpatient stay and genetics consultation occurred between December 1, 2011 and June 30, 2017

ES-cohort patients met the following additional criteria:

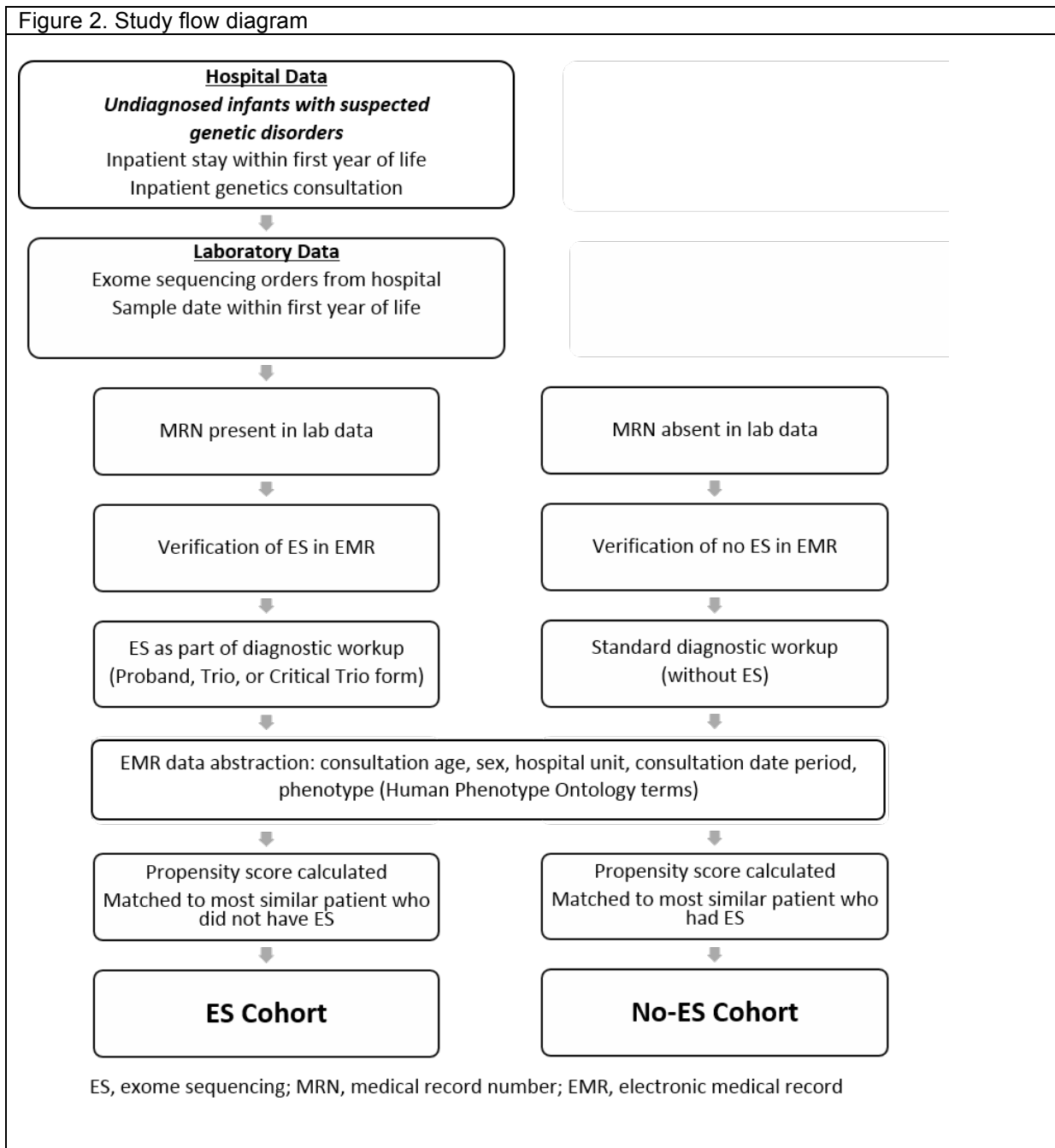
- Received ES (proband-only, trio, or critical trio) as part of the diagnostic pathway
- ES ordered within the first year of life

Among all included patients who met the above criteria, patients eligible for inclusion in the No-ES cohort were defined as patients who did not receive ES in the diagnostic pathway. They may have received other forms of genetic testing such as single gene or gene panel tests (e.g., cardiomyopathy panel, BluePrint panel, CHD7 sequencing, Noonan panel, SMA panel), chromosomal microarray, and methylation studies. A clinician may have ordered ES for patients in the No-ES cohort without it being performed for reasons such as lack of parental consent or cancelation by lab due to insufficient blood sample.

Two datasets were combined to define the patient population. The first dataset, obtained from the hospital, contained medical record numbers of all patients at TCH who (1) had an intensive care unit inpatient admission within the first year of life, and (2) had an order for inpatient consultation from the Genetics service. The second dataset, obtained from the diagnostic laboratory, contained ES report data for all patients who had ES (1) ordered from TCH, and (2) an ES order date less than 366 days from date of birth. All ES ordered at TCH is sent to BG. Datasets were merged on medical record number. Patients appearing in both datasets were preliminarily designated as the ES cohort; patients appearing only in the hospital

data were preliminarily designated as the No-ES cohort, subject to verification of inclusion criteria via EMR review.

Figure 2. Study flow diagram



EMR review was then performed for all patients. We defined the index admission as the admission during which the initial Genetics consult was ordered. Patients for whom a consult order was placed but later canceled were not included. A consult order and a note from a member of the Genetics service (even if not electronically filed as a “consult”) indicated consultation.

Patients in the ES cohort had one of 3 forms of ES: sequence analysis of only the patient (proband), a trio of patient and both parents (trio), or trio of patient and parents with expedited turnaround time (critical trio). Clinical ES became available in October 2011 in proband form. The trio test was introduced in October 2014 and critical trio in April 2015.

To determine comparable cohorts of patients who did and did not have ES, we calculated a propensity score for each patient. This method allows adjustment for confounding when assessing multiple outcomes.<sup>85,86</sup> The propensity score was used to represent multiple HPO terms and other relevant factors as a one-dimensional score.<sup>87</sup> We generated a binary variable for each HPO identification (ID) number appearing in the data. HPO ID numbers were used instead of terms themselves to ensure synonymous terms did not appear as separate variables. Granularity was preserved; we did not use hierarchical processing to map to higher order terms (although in many cases, multiple levels of terms were generated). HPO term variables with count fewer than 10, meaning that the term was observed in fewer than 10 patients, were dropped, leaving 340 term variables. Based on clinician consensus, another 33 term variables were dropped because they related to transient clinical characteristics, such as fever or emesis, not relevant for making a



diagnosis. HPO terms were selected for inclusion in the propensity score model using a backward automated variable selection process ( $p$ -value for removal = 0.1).

We calculated a propensity score, which is the estimated probability of having ES conditional on measured covariates, for each patient using a binary logistic regression model with ES as the dependent variable that included indicator variables for: gender, the unit in which the initial Genetics consult was performed, initial Genetics consult date (quartile of study period), age (days) at first genetics consult (quarter of year), and HPO terms. After predicting propensity score for each patient, each ES patient was matched to one most phenotypically similar No-ES patient from among all potential No-ES patients using a greedy matching algorithm based on the linear predictor of the propensity score. Compared to differences between ES patients and the entire group of No-ES patients prior to matching, the matching procedure successfully reduced differences in covariates between ES and No-ES patients in the final cohorts.

## **Study Power**

### ***Aim 1***

Not Applicable.

### ***Aims 2 and 3***

The sample size in this study was fixed and unknown prior to completion of the data collection process. The statistical power to detect a difference in outcomes between study arms was calculated prior to performing any data collection. The

most relevant information to calculate power was from a study of infants admitted to an ICU at TCH in the first 100 days of life who had ES.<sup>3</sup> The 120-day mortality for patients in the sample was 81/272 (29.8%). The study does not provide any information on patients who did not have ES.

The ability to detect a difference in effect between a diagnostic pathway that includes ES and a diagnostic pathway that does not include any form of ES is of interest. Because this study will utilize matched pairs and 120-day mortality is a dichotomous outcome, the power calculation is performed for a two-sample paired-proportions test (McNemar's). Assuming a 120-day mortality of 29.8%, sample size of 400 pairs, and sum of discordant proportions of 0.50, the power would be 77.7% to detect a 9.6% difference in 120-day mortality using a two-sided test and alpha of 0.05. In the smallest expected study arm (trio ES), assuming a sample size of 50 patients and all of the above conditions, the power would be 15.8%.

The detectable effects size as an odds ratio at a desired power level of 0.80 can also be calculated. With 400 matched sets of cases and controls and one matched control per case, assuming a 120-day mortality of 0.298 and a correlation coefficient for mortality between matched cases and controls of 0.50, and alpha of 0.05, a true odds ratios for impact of ES in patients who died relative to patients who did not die of 0.576 or 1.615 would be detectable with a power of 0.80. Similarly, with 50 matched sets, true odds ratios for impact of trio ES of .139 or 3.519 would be detectable with power of 0.80.

## **Data Collection**

### ***Aim 1***

Key pieces of information were collected from each included article. A data extraction form was developed and pilot tested, and then two refined versions were created based on the two types of analyses and reporting encountered. For the purpose of collecting and presenting results in this review, studies of five or fewer patients were considered “case reports” and studies of more than five patients were considered “aggregate analyses.” The cutoff number of five was determined based on differences in article structure and information presentation according to the number of patients included. Thus, the data collection form used for each type of study reflected the way in which facts were reported.

Data items selected for abstraction from articles were broadly based on parameters recommended for assessment in evaluation of genetic tests.<sup>88</sup> The data collection form for aggregate analyses included the following items: study objective, country, type of CGS, comparator, clinical setting, study design, outcome measures, study population, inclusion criteria, exclusion criteria, average age at test, percent of probands younger than 19 years of age, percent of probands who were male, diagnostic laboratory, sequencing platform, whether a duo and/or trio approach was used, turnaround time, molecular diagnostic yield, number of probands with a change in medical management, discussion of insurance coverage, discussion of costs or cost-effectiveness, and average cost to diagnosis or cost of potentially replaced tests. For case reports, the above information was collected on the individual level as well as the gene implicated and diagnosis. For economic studies,

the perspective of the analysis, cost data source, and incremental cost per outcome measure were recorded. Data from all included studies was abstracted into a spreadsheet. Analysis was performed with Stata IC 13 (College Station, Texas). Variables to be measured are presented in Table 2.

Table 2. Measurement Matrix for Aim 1		
Variable	Definition	Coding Scheme
Publication Year	Year article was published	Numeric
First Author	Name of first author of article	Text
Title	Title of Article	Text
First Author Geneticist	Indicator of whether the first author was a geneticist	1 = First author is a geneticist 0 = Otherwise 888 = Cannot determine
Study Objective	Stated objective of article	Text
Country of Origin	Country of the clinical setting	Text
Study Location	Name of hospital or clinic (or description if name not given)	Text
Study Dates	Beginning and end date of study data collection period	Text
Center	Single versus multicenter study	1 = Single Center 2 = Multi-center
Type of NGS	ES, WGS	Text
Comparator	What NGS diagnostic tool is compared to	Text
Clinical Setting	NICU, PICU, clinic, lab, etc.	Text
Study Design	Description of study design	Text

Table 2. Measurement Matrix for Aim 1		
Variable	Definition	Coding Scheme
Outcome Measures Listed (explicitly as goal of study)*	Whether outcome measures are listed in the text of the article	1 = Yes 0 = No
Outcome Measures Defined*	Whether an operational definition of outcome measures is provided in the text	1 = Yes 0 = No
Outcome Measures	Outcome measures reported	Text
Study Population	Description of study population	Text
Inclusion Criteria	Description of inclusion criteria	Text
Exclusion Criteria	Description of exclusion criteria	Text
Sample Size	Total sample size of individuals sequenced	Numeric
Number of Patients*	Total number of patients included	Numeric
Number of Families*	Total number of families included	Numeric
Age at Test	Average age at test date (record in units given)	Text (include unit in parentheses)
% of Patients < 18 y.o.*	Percent of patients less than 18 years of age at test date	Numeric 888 = Cannot determine 999 = Not Given
Sex (% Male)	Percent of patients sampled who were male	Numeric 888 = Cannot determine 999 = Not Given
Lab	Name of laboratory that performed NGS (may be more than one)	Text (e.g., BG, Ambry, GeneDX)
Multi-lab*	Whether samples were sent to more than one lab	1 = More than one lab used 0 = All samples sent to

Table 2. Measurement Matrix for Aim 1		
Variable	Definition	Coding Scheme
		single lab
Platform	Sequencing platform used by the lab	Text (e.g., Illumina HiSeq 2500)
Consensus review*	Whether a clinical consensus review process for discussing laboratory results is described	1 = Clinical team consensus review of results described 0 = No clinical team consensus review of results described 2 = review by ordering geneticist
Use ACMG Variant Pathogenicity Category	Whether ACMG variant pathogenicity categories are used to categorize findings	1 = ACMG categories used 0 = ACMG categories not used 999 = Not Given
Trio Approach	Whether a trio approach was used to also sequence parents or siblings	1 = Trio approach used in at least some cases 0 = Trio approach never used
Trio Preferred*	Whether trio approach was preferred/recommended, as indicated by the authors	1 = Trio approach preferred/recommended 0 = Trio approach not preferred/recommended

Table 2. Measurement Matrix for Aim 1		
Variable	Definition	Coding Scheme
Confirmation by Sanger Sequencing*	Whether confirmation of variants by Sanger sequencing was performed	1 = Confirmation by Sanger sequencing 0 = No confirmation by Sanger sequencing 999 = Not Given
Turnaround time	Average turnaround time of test (record in units given)	Numeric
Report Incidental Findings	Whether "incidental" or "secondary" findings unrelated to clinical phenotype are reported	1 = Reported 0 = Not reported 999 = Not Given
Percent of Patients with Incidental Findings	Percent of patients who had incidental findings returned	Numeric
Molecular diagnostic yield*	Overall molecular diagnostic yield (%)	Numeric 888 = Cannot determine 999 = Not Given
Diagnostic‡	Diagnostic outcome of sequencing	0 = Non-diagnostic 1 = Diagnostic 2 = Prompted candidate gene association studies 3 = Most likely candidate for clinical presentation
Phenotypic subgroups reported*	Whether analysis is broken down into clinical phenotype subgroups	1 = Yes 0 = No
Phenotype‡	Description of patient's phenotype	Text

Table 2. Measurement Matrix for Aim 1		
Variable	Definition	Coding Scheme
Neurologic phenotype (%)*	Percent of patients classified as a neurologic phenotype	Numeric 999 = Not Given
Neurologic phenotype diagnostic yield (%)*		
Congenital structural anomaly (%)*	Percent of patients with a congenital structural anomaly	Numeric 999 = Not Given
Congenital structural anomaly diagnostic yield (%)*		
Change in Medical management*	Percent of patients with a reported change in medical management following return of sequencing results	Numeric 999 = Not Given
Change in Medical management description‡	Change in medical management for the proband described in the case	Text
Description of change in medical management*	Ways in which authors report that medical management was changed following return of sequencing results	Text
Other health outcome measure*	Health outcome metric other than those collected in this form	Text
Discussion of medical management change	Authors discuss that sequencing results may impact medical management of the patient	1 = Yes 0 = No
Discussion of economics	Authors discuss economic notions related to sequencing	1 = Yes 0 = No
Discussion of economic	Authors discuss challenges related to health	1 = Yes



Table 2. Measurement Matrix for Aim 1		
Variable	Definition	Coding Scheme
evaluation methodology challenges*	economic evaluation of genetic sequencing	0 = No
Average cost to diagnosis	Average cost to diagnosis via sequencing	Numeric . = Not Given
Cost of potentially replaced tests	Collective cost of potentially avoided diagnostic testing if sequencing used as a first-line test	Numeric . = Not Given
Discussion of insurance coverage	Authors discuss insurance coverage of clinical genomic sequencing	1 = Yes 0 = No
Strength	Study strengths from study design perspective	Text
Limitations	Study limitations from study design perspective	Text
*Aggregate analyses only		
‡ Case Reports only		

### ***Aims 2 and 3***

ES uptake and health outcomes were systematically assessed through retrospective EMR review. A data collection form was designed to collect key pieces of information from the TCH Epic EMR system. Each patient's EMR was individually reviewed, and data were extracted and compiled in a spreadsheet with a single row of data for each patient that combined EMR and BG data. EMR review was

completed in August 2018 such that there is at least one year of clinical follow-up data on all patients.

Variables relevant for matching were collected for all patients who met the inclusion criteria (cases and potential controls). To address heterogeneity of the patient population, subgroup analyses were performed. Clinically relevant features were used to define the strata. Patients were divided into subgroups intended to generate evidence to inform clinical decision-making and guideline development. We performed subgroup analyses for patients admitted in 2016 and 2017 after all three ES forms were available, patients who survived to 28 days of life, patients in the NICU during the genetics consultation, and patients in the ES-recommended group.

Medical records were filtered for diagnostic-related activities performed during the patient's ICU stay and over the year following the initial Genetics consultation and clinical notes originating from a member of the genetics service (inpatient) or genetics clinic (outpatient), including notes signed by geneticists, genetics trainees, and genetic counselors.

Cost data was obtained from the hospital administrative cost reports. Patient-level costs were reported for the index admission and over the year following the initial genetics consult order date. As there is no standard diagnostic pathway for this population of patients, we defined the diagnostic pathway as clinical tests performed for the purpose of making a diagnosis, rather than routine care or monitoring. An inclusion rule of "first," "none," or "all" was determined for each test on a list of all laboratory and radiology tests performed in our study sample. The rule was used to determine which, if any, instance of a specific test in a patient was counted as part of

the diagnostic pathway and applied to the cost data to sum the cost of diagnostic pathway investigations. Similarly, to determine the cost of genetic tests, we identified each genetic test in the list of laboratory tests. In each patient's cost data, each line-item for "miscellaneous referred test" was cross-referenced by service date to miscellaneous referred tests ordered in the EMR to determine whether it was a genetic test or not. All tests determined to be genetic tests were included in both the diagnostic pathway and genetic test cost categories. Genetic test and diagnostic pathway costs are not necessarily inclusive or exclusive of billing categories.

Variables to be measured for Aim 2 and Aim 3 are presented in Table 3 and Table 4, respectively.

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Patient Characteristics			
ES	Indicates whether patient had ES	0 = Patient did not have ES 1 = Patient had ES	BG; TCH Administrative Records
Last name	Patient last name	Text	TCH Administrative Records
Date of Birth	Patient date of birth	Date	TCH Administrative Records

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
MRN	Medical record number	Numeric	TCH Administrative Records
First IP Genetics consult order date	Date first order for genetics consult during ICU stay was placed in TCH EMR from TCH records	Date	TCH Administrative Records
Ethnicity	Ethnicity of patient as listed in Demographics tab in TCH EMR (or from H&P or genetics note if not listed in Demographics. If genetics note in conflict with demographics tab facesheet, deferred to genetics note)	Hispanic Non-Hispanic unknown	TCH EMR
Race	Race of patient as listed in Demographics tab in TCH EMR (or from H&P note if not listed in Demographics)	American Indian and Alaska Native Asian Black/African-American Native Hawaiian or Other Pacific Islander White/Caucasian unknown	TCH EMR
Sex	Patient sex as listed in TCH EMR	M = male	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
		F = female U = ambiguous ("none")	
Alive	Indicator of whether a patient is alive as determined in TCH EMR through review of notes. Note that even if a patient is not marked as deceased in the TCH EMR system, documentation of communication of patient death was used to determine status.	0 = Deceased 1 = Alive	TCH EMR
Primary Language	Primary language of family as listed in TCH EMR or as indicated in clinical notes was spoken with the family (or spoken through translator). If at least one member of the family could communicate with medical staff in English, and no translator used, recorded as English.	English Spanish Other	TCH EMR
Zip code	Patient zip code, identified from Detailed Report for admission from index admission	Numeric	TCH EMR
Date of last follow-up or death	Date of last follow up or death as determined from encounter list in TCH EMR	Date	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Date of Death	Date of death as determined in TCH EMR from death summary note or other note	Date	TCH EMR
Date ICU admit	Date of index ICU admission (defined as admission during which initial inpatient genetics consult order was placed), identified from Detailed Report for admission	Date	TCH EMR
Length of inpatient stay	Length of inpatient stay in days for index ICU admission (defined as admission during which initial inpatient genetics consult order was placed), identified from Detailed Report for admission	Numeric	TCH EMR
Date ICU discharge	Date of discharge for index ICU admission (defined as admission during which initial inpatient genetics consult order was placed), identified from Detailed Report for admission	Date	TCH EMR
Discharge to	Place where patient was discharged to following index ICU admission, identified from Detailed Report for admission as "Disposition"	Expired Home Health Care Home/Self Care Hospice Other facility	TCH EMR
Admit Service	Service listed under admission	Text	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
	information, identified from Detailed Report for admission		
Principal problem	Principal clinical problem for admission, identified from Detailed Report problem list as item with blue square	Text	TCH EMR
Principal problem_ICD-10-CM	ICD-10-CM code for principal problem for admission, identified from Detailed Report problem list as item with blue square	Text	TCH EMR
Reason for visit_DXcode1	Dx code for Reason for Visit (coded), identified from Detailed Report for admission	Text	TCH EMR
Reason for visit_DX1	Diagnosis for Reason for Visit (coded), identified from Detailed Report for admission	Text	TCH EMR
Reason for Admission - Primary	Primary reason for admission identified from Detailed Report for admission	Text	TCH EMR
Reason for admission - Primary_code	ICD-9 (or ICD-10) code corresponding to the primary reason for admission identified from Detailed Report for admission	Text	TCH EMR
Clinical setting (unit)	Unit listed in Detailed Report for admission	Text	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Insurance Payer_Epic (priority 1)	Insurance carrier in TCH EMR (at time of admission) - priority 1	Text	TCH EMR
Insurance Payer_Epic (priority 2)	Insurance carrier in TCH EMR (at time of admission) - priority 2	Text	TCH EMR
Point of Origin	Place where patient was admitted from for index ICU admission, identified from Detailed Report for admission as "Point of Origin"	Clinic or Physician Referral Newborn at TCH Self Referral/Non-Health Care Facility Transfer Center	TCH EMR
Gestational age	Gestational age at birth, identified from H&P, Genetics, or discharge note	Numeric	TCH EMR
Age of mother (years)	Age of mother at patient's birth, identified from H&P or Genetics note	Numeric	TCH EMR
Mother Parity	G&P status of mother prior to delivery of patinet, identified from H&P or Genetics note	Text	TCH EMR
Primary Caregiver marital status	Primary caregiver's marital status, identified from Social Work, H&P, or other notes	Living with partner Engaged Married	TCH EMR



Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
		Separated Single Unknown	
Age of father (years)	Age of father at patient's birth, identified from H&P or Genetics note	Numeric	TCH EMR
Unit_Genetics consult note	Unit listed on initial inpatient Genetics consult note	Text	TCH EMR
Genetics Consult Note Chief Complaint	Chief complaint listed on initial Genetics Consult Note	Text	TCH EMR
Initial Genetics Consult Note Author	Author of initial Genetics Consult Note (Fellow or Resident)	Text	TCH EMR
Initial Genetics Consult Note Cosigner	Cosigner of initial Genetics Consult Note (Attending)	Text	TCH EMR
Date of initial Genetics Consult	Date of initial inpatient Genetics Consult	Date	TCH EMR
ES Genetics Consult/Physician Note Author	Author of Genetics Consult Note describing ES order (inpatient only) (Fellow or Resident)	Text	TCH EMR
ES Genetics Consult/Physician	Cosigner of Genetics Consult Note describing ES order (inpatient only)	Text	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Note Cosigner	(Attending)		
Date of ES Genetics Consult	Date of Genetics Consult that describes ES order (inpatient only)	Date	TCH EMR
ES ordered during different admission than initial Genetics consult	Whether ES was ordered during a different inpatient admission than the index admission (inpatient only; outpatient = 0)	0 = No (ES ordered during index admission or outpatient) 1 = Yes	TCH EMR
Principal Problem for admission during ES ordered if different	Principal problem for admission during which ES was ordered if ordered during a different admission than the index admission	Text	TCH EMR
Date of Admit for ES admission (if different from index)	Date of admission for inpatient stay during which ES was ordered if different than index admission	Date	TCH EMR
Date of Discharge for ES admission (if different from index)	Discharge date for inpatient stay during which ES was ordered if different than index admission	Date	TCH EMR
ES Test Code	ES test code	1500 = Proband 1600 = Trio 1722 = Critical	BG

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
		Trio	
Date ES Ordered_Epic	Date ES was ordered in TCH EMR, as listed on the order	Date	TCH EMR
Date ES Resulted_Epic	Date ES was resulted in TCH EMR, as listed on the order	Date	TCH EMR
Date ES Results Reviewed_Epic	Date ES results were viewed in TCH EMR the first time, as listed on the order	Date "not listed" = no reviewer name/date	TCH EMR
Date ES Results Reviewed 2_Epic	Date ES results were viewed in TCH EMR the second time, as listed on the order	Date	TCH EMR
ES result addendum/Expanded report	Whether there was an addendum, expanded report, or reanalysis document scanned into the ES order in TCH EMR	0 = No 1 = Yes	
ES result pending at discharge	Whether ES results were pending at ICU discharge for admission during which initial Genetics consult was ordered. Note: always = 1 if ES was ordered after index admission.	0 = Returned during inpatient admission during which first Genetics consult occurred 1 = ES result not returned during admission during	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
		which first Genetics consult was ordered	
ES Order Clinical Setting	Clinical setting in which ES was ordered	Inpatient Outpatient	TCH EMR
ES ordering physician if outpatient	Name of physician who ordered ES if ordered outpatient	Text	TCH EMR
Date ES recommended if outpatient	Date that physician recommended ES if ordered outpatient. Note: sometimes a lag before order placed because of insurance authorization process	Date	TCH EMR
Result Return Geneticist	Name of Genetics Service provider who returned ES results to family	Text	TCH EMR
Result Return Geneticist_Atending	Name of Genetics Service provider who returned ES results to family - attending geneticist	Text	TCH EMR
Result Return Geneticist_Fellow/Resident	Name of Genetics Service provider who returned ES results to family - genetics fellow or resident	Text	
Result Return Other Provider	Name of Genetics Service provider who returned ES results to family - specialty other than Genetics	Text	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Result Return Counselor	Name of Genetic Counselor who returned ES results to family	Text	TCH EMR
ES Result Return mode	Mode of delivery of ES results to family	Telephone Inpatient Genetics Clinic Visit Neurology Clinic Visit Metabolic Clinic Visit Other	TCH EMR
ES Result communication (first contact)	Date of first communication of ES results with patient's family	Date	TCH EMR
ROR Notes	Notes about how return of results was performed	Text	TCH EMR
Genetic Counseling Notes	Notes from genetic counseling result disclosure	Text	TCH EMR
Follow Up in Genetics Clinic_date	Date patient had in-person follow-up visit in the Genetics outpatient clinic	Date	TCH EMR
Follow Up in Genetics	Clinician who saw patient in Genetics outpatient clinic at follow-up	Text	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Clinic_MD			
Control_ES noted recommended or reflex	For control patients only; whether the Genetics consult note had clinician documentation in the plan that they would consider ES in the future or reflex to it	1 = Yes . = No/not noted	TCH EMR
Parents decline ANY genetic testing	For control patients only; whether the Genetics consult note had clinician documentation that the patient's parents declined having any genetic testing	1 = Yes . = No/not noted	TCH EMR
MedicalRefNo_BG	MRN from BG records	Numeric	BG
TestCode_BG	Test code from BG records	1500 = Proband 1600 = Trio 1722 = Critical Trio	BG
MedicalPresentation_BG	Medical presentation as described by the clinician and given to the lab	Text	BG
SampleDate_BG	ES sample date from BG records	Date	BG
TestOrderDate_BG	ES order date from BG records	Date	BG
Focused Report Date	Date focused report was faxed from BG	Date	TCH EMR (retrieved from scans of BG reports in TCH)

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
			EMR)
Last_FaxedDate_BG	ES result fax date from BG records. Date last report (expanded, addendum, reanalysis, etc.) was faxed. If no reanalysis, same as date of focused report.	Numeric	BG
TestTurnAround (days, FaxedDate - OrderDate)_BG	ES turnaround time (days)		BG
ClinicalSummary_BG	Clinical summary from BG	Text	BG
Interpretation_BG	Interpretation of ES results from BG	Text	BG
Clinician Interpretation of Results	Interpretation of Geneticist who followed up with patient after ES results available from clinic notes	Remarkable Unremarkable	TCH EMR
HPO terms_curated	Curated list of HPO terms generated for the patients via natural language processor from genetics consultation, admission, and discharge notes	Text	TCH EMR
Outcome Measures			
Molecular diagnostic result	Molecular diagnosis	Text	TCH EMR
Molecular	Description of molecular diagnosis	Text	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
diagnosis description			
Molecular diagnostic tool	Diagnostic test used to determine molecular diagnosis	Text	TCH EMR
Parent Availability note	Description of family members who submitted samples to the lab for testing		BG
Clinical Diagnosis	Clinical diagnosis (may or may not be molecularly confirmed)	Text	TCH EMR
CMA Completed	Indicator of whether patient had chromosomal microarray (CMA), either at TCH or outside hospital (as documented in clinical note)	0 = No CMA performed 1 = CMA performed	TCH EMR
CMA Result	Result of CMA	Normal Gain Loss AOH (absence of heterozygosity)	TCH EMR
Other genetic tests	List of other genetic tests performed, as collected from Results Review tab in EMR	Text	TCH EMR
Change in counseling	Whether there was a change in counseling following ES results	0 = No change in counseling 1 = Change in counseling	TCH EMR



Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Change in counseling notes	Notes about change in counseling	Text	TCH EMR
Sub-specialty consult	Whether there was a sub-specialty consult initiated following ES results	0 = No surgical intervention following molecular diagnosis from ES 1 = Surgical intervention following molecular diagnosis from ES	TCH EMR
Sub-specialty consult notes	Notes about sub-specialty consult	Text	TCH EMR
Change in medication	Whether there was a change in drug therapy initiated following ES results	0 = No change in drug therapy (initiation or discontinuation) following molecular diagnosis from ES 1 = Change in drug therapy	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
		(initiation or discontinuation) following molecular diagnosis from ES	
Change in medication notes	Notes about change in drug therapy	Text	TCH EMR
Surgery/Procedure	Whether there was a surgery or procedure performed following ES results	0 = No surgical intervention following molecular diagnosis from ES 1 = Surgical intervention following molecular diagnosis from ES	TCH EMR
Surgery/Procedure notes	Notes about surgery or procedure		TCH EMR
Change in screening	Whether there was a change in screening following ES results	Text	TCH EMR
Change in screening notes	Notes about change in screening	Text	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Change in diet	Whether there was a change in diet initiated following ES results	0 = No change in diet 1 = Change in diet	TCH EMR
Change in diet notes	Notes about diet change	Text	TCH EMR
Change in Prognosis	Whether there was a change in prognosis based on ES results	Text	TCH EMR
Change in Prognosis notes	Notes about prognosis change	Text	TCH EMR
Palliation	Whether palliation was initiated following ES results	0 = Palliation not initiated 1 = Palliation initiated	TCH EMR
Palliation notes	Notes about palliation	Text	TCH EMR
BMT	Whether there was a bone marrow transplant initiated following ES results	0 = No BMT following molecular diagnosis from ES 1 = BMT following molecular diagnosis from ES	TCH EMR
BMT notes	Notes about BMT	Text	TCH EMR
Surveillance Plan	Whether there was a change in	0 = No change in	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
	surveillance plan initiated following ES results	surveillance plan 1 = Change in surveillance plan	
Surveillance plan notes	Notes about surveillance plan	Text	TCH EMR
Inheritance pattern	Whether there was a change in thinking about inheritance pattern of disease following ES result return	0 = No change in inheritance pattern thinking 1 = Change in inheritance pattern thinking	TCH EMR
Inheritance pattern notes	Notes about natural history	Text	TCH EMR
Additional Genetic Testing of Proband	Whether additional genetic testing of proband was recommended after ES results received	0 = No additional genetic testing of proband recommended 1 = Additional genetic testing of proband recommended	TCH EMR
Additional Genetic Testing of Proband	Notes regarding additional genetic testing of proband recommended after ES results	Text	TCH EMR

Table 3. Measurement Matrix for Aim 2			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
notes	received		
Family testing/surveillance	Whether ES results prompted a recommendation of genetic testing or medical surveillance in the proband's family members	0 = No family testing/surveillance 1 = Family testing/surveillance recommended	TCH EMR
Family testing/surveillance notes	Notes about family testing/surveillance	Text	TCH EMR
Other clinical care effect	Notes about other effects of ES on clinical care	Text	TCH EMR

Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Cost Categories			
Index admission total cost	Total cost of index admission	Continuous	TCH Administrative Records
Year post encounters	Count of encounters over year following initial genetics consult	Discrete	TCH Administrative Records
Year post total	Total cost of one year following index admission	Continuous	TCH

Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
cost			Administrative Records
Index and year encounters	Count of index admission + encounters over year following initial genetics consult	Continuous	TCH Administrative Records
Index and year total cost	Total cost combined (index admission and one year following)	Continuous	TCH Administrative Records
Day Surgery Count Post	Number of post index admissions encounters - day surgery	Discrete	TCH Administrative Records
Emergency Count Post	Number of post index admissions encounters - emergency	Discrete	TCH Administrative Records
Inpatient Count Post	Number of post index admissions encounters - inpatient	Discrete	TCH Administrative Records
Observation Count Post	Number of post index admissions encounters - observation	Discrete	TCH Administrative Records
Outpatient Count Post	Number of post index admissions encounters - outpatient	Discrete	TCH Administrative Records

Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Renal Series Count Post	Number of post index admissions encounters - renal series	Discrete	TCH Administrative Records
Series Count Post	Number of post index admissions encounters - series	Discrete	TCH Administrative Records
Specimen Count Post	Number of post index admissions encounters - specimen	Discrete	TCH Administrative Records
Clinic Index Cost	Index admission cost by UB revenue code category - clinic	Continuous	TCH Administrative Records
Diagnostic Index Cost	Index admission cost by UB revenue code category - diagnostic	Continuous	TCH Administrative Records
Emergency Care Index Cost	Index admission cost by UB revenue code category - emergency care	Continuous	TCH Administrative Records
Laboratory Index Cost	Index admission cost by UB revenue code category - laboratory	Continuous	TCH Administrative Records
Med/Surg Supplies Index	Index admission cost by UB revenue code category - med/surg supplies	Continuous	TCH Administrative

Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Cost			Records
Nursing Care Index Cost	Index admission cost by UB revenue code category - nursing care	Continuous	TCH Administrative Records
OR Periop Index Cost	Index admission cost by UB revenue code category - OR periop services	Continuous	TCH Administrative Records
Organ Acq Index Cost	Index admission cost by UB revenue code category - organ aq	Continuous	TCH Administrative Records
OT/PT Index Cost	Index admission cost by UB revenue code category - OT/PT	Continuous	TCH Administrative Records
Other Index Cost	Index admission cost by UB revenue code category - other	Continuous	TCH Administrative Records
Pharmacy Index Cost	Index admission cost by UB revenue code category - Pharmacy	Continuous	TCH Administrative Records
Radiology Index Cost	Index admission cost by UB revenue code category - radiology	Continuous	TCH Administrative Records
Therapeutic	Index admission cost by UB revenue code	Continuous	TCH



Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Index Cost	category - therapeutic		Administrative Records
Transport Index Cost	Index admission cost by UB revenue code category - transport	Continuous	TCH Administrative Records
Clinic Post Cost	Post-index admissions' cost by UB revenue code category - clinic	Continuous	TCH Administrative Records
Diagnostic Post Cost	Post-index admissions' cost by UB revenue code category diagnostic	Continuous	TCH Administrative Records
Emergency Care Post Cost	Post-index admissions' cost by UB revenue code category - emergency care	Continuous	TCH Administrative Records
Laboratory Post Cost	Post-index admissions' cost by UB revenue code category - laboratory	Continuous	TCH Administrative Records
Med/Surg Supplies Post Cost	Post-index admissions' cost by UB revenue code category - med/surg supplies	Continuous	TCH Administrative Records
Nursing Care Post Cost	Post-index admissions' cost by UB revenue code category - nursing care	Continuous	TCH Administrative Records

Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
OR Periop Services Post Cost	Post-index admissions' cost by UB revenue code category - OR periop services	Continuous	TCH Administrative Records
Organ Acquisition Post Cost	Post-index admissions' cost by UB revenue code category - organ acquisition	Continuous	TCH Administrative Records
OT/PT Post Cost	Post-index admissions' cost by UB revenue code category - OT/PT	Continuous	TCH Administrative Records
Other Post Cost	Post-index admissions' cost by UB revenue code category - other	Continuous	TCH Administrative Records
Pharmacy Post Cost	Post-index admissions' cost by UB revenue code category - pharmacy	Continuous	TCH Administrative Records
Radiology Post Cost	Post-index admissions' cost by UB revenue code category - radiology	Continuous	TCH Administrative Records
Therapeutic Post Cost	Post-index admissions' cost by UB revenue code category - therapeutic	Continuous	TCH Administrative Records
Transport Post Cost	Post-index admissions' cost by UB revenue code category - transport	Continuous	TCH Administrative

Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
			Records
Index admission diagnostics count	Count of tests on the "diagnostic pathway" during the index admission	Discrete	TCH Administrative Records
Index admission diagnostics total cost	Total cost of diagnostic pathway (using "first" and "all" rules) for the index admission	Continuous	TCH Administrative Records
Year diagnostics count	Count of tests on the "diagnostic pathway" from the beginning of the index admission through one year after the initial genetics consult	Discrete	TCH Administrative Records
Year diagnostics total cost	Total cost of diagnostic pathway (using "first" and "all" rules) for the year	Continuous	TCH Administrative Records
Index admission genetic test total cost	Total cost of genetic tests during the index admission	Continuous	TCH Administrative Records
Year genetic test total cost	Total cost of genetic tests during the index year	Continuous	TCH Administrative Records
Measured Variable	Variable Description	Variable Coding/Type	Data Source for calculation
Variables Used to Perform Subgroup analyses			
Patient Characteristics			

Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
Proband-only	Patient had BG test code 1500	Categorical (0/1)	BG
Trio ES	Patient had BG test code 1600	Categorical (0/1)	BG
Critical trio ES	Patient had BG test code 1722	Categorical (0/1)	BG
No ES	Patient did not have ES as part of diagnostic pathway (No-ES Cohort)	Categorical (0/1)	TCH EMR
NICU	Patient had initial genetics consultation in the NICU	Categorical (0/1)	TCH EMR
Survived to 28 days	Patient survived to 28 days of life	Categorical (0/1)	TCH EMR
Survived to 1 year of life	Patient survived to 1 year of life	Categorical (0/1)	TCH EMR
Admit year	Year of admission date for index admission	2011 2012 2013 2014 2015 2016 2017	TCH EMR
Outcome Measures			
Molecular	Whether a molecular diagnosis was made	Categorical	TCH EMR

Table 4. Measurement Matrix for Aim 3			
Measured Variable	Variable Description	Variable Coding/Type	Data Source
diagnosis		(0/1)	
28-day survival	Patient survived to 28 days of life	Categorical (0/1)	TCH EMR
1-year survival	Patient survived to 1 year of life	Categorical (0/1)	TCH EMR

## Data Analysis

### *Aim 1*

Because this scoping review included articles that employed multiple methodologies and studied diverse patient populations, results across studies were summarized and narratively described rather than combined statistically in a meta-analysis. Descriptive statistics were calculated on the number of articles on each type of CGS, characteristics of patients and institutions, clinical scenarios, and reported outcome measures. Discussion of costs and economic evidence was also summarized. The Consolidated Health Economic Evaluation Reporting Standards (CHEERS) checklist was used to assess the quality of reporting in articles with an economic evaluation focus.<sup>89</sup> Two authors assessed each article independently and arrived at a consensus score.

## ***Aim 2***

Data management and descriptive analyses were performed using Stata/IC 15 (StataCorp, College Station, TX). Detailed electronic medical record review was performed for all patients who met inclusion criteria. The index admission was defined as the admission during which the initial genetics consult was ordered and the index year as the year following the date of initial genetics consultation.

To characterize patient phenotypes, relevant information was extracted from the initial Genetics consult note (which contains the most extensive and detailed assessment of the patient's clinical features) and index admission discharge note. A natural language processor was used to generate human phenotype ontology (HPO) terms based on the clinical characteristics of the patients described in notes. Thus, the set of HPO terms generated for each patient was intended to capture clinical presentation at the time of the Genetics team's assessment. Careful attention was given so as not to include "pertinent negatives," information from birth or family history, or phenotypic hallmarks of differential diagnoses described within the note.

A binary variable was generated for each HPO ID number appearing in the data. HPO ID numbers were used to ensure synonymous terms did not appear as separate variables. Granularity was preserved; we did not use hierarchical processing to map to higher order terms (although in many cases, both were generated). HPO term variables with count fewer than 10 (i.e., terms that were observed in fewer than 10 patients) were dropped, leaving 340 term variables. On the basis of consensus among clinicians, another 33 term variables were dropped

because they related to transient clinical characteristics, such as fever or emesis, not relevant for making a diagnosis.

A propensity score was then calculated for each patient. The propensity score is the estimated probability of having ES conditional on measured covariates. It is used to represent multiple HPO terms and other relevant factors as a one-dimensional score.<sup>87</sup> Propensity scores may be used to adjust for confounding when assessing multiple outcomes.<sup>85,86</sup> The propensity score was calculated using a binary logistic regression model that included indicator variables for: the department in which the initial Genetics consult was performed, quartiles of time since ES availability, age at first genetics consult by quarter of year, and patient gender. A backward automated variable selection process (p-value for removal = 0.1) was used for selection of HPO term variables. Patients who had ES were matched to phenotypically similar patients who did not have ES using a greedy matching algorithm based on the natural log of the propensity score. Compared to differences between ES patients and the entire group of No-ES patients prior to matching, the matching procedure successfully reduced differences in covariates between ES and No-ES patients in the final cohorts.

We calculated descriptive statistics on demographics of patients and characteristics of the index admission. We produced Kaplan-Meier survival curves to analyze survival to 28 days, 1 year, and to the end of study. We used Cox regression models to analyze survival times and logistic regression models to analyze odds of molecular diagnosis.

Outcomes were analyzed using the appropriate statistical test for the nature of the data involved to test for a difference in outcome and costs between the study arms and for subgroup analyses (e.g., Student's t-test, Chi-square test, Wilcoxon rank-sum and Kruskal-Wallis tests for differences in cost categories between cohorts and ES forms, respectively.)

Effects were tallied from the clinical note at the time of ES return of results and follow-up in the Genetics clinic. Establishment of a molecular diagnosis and survival were the primary outcomes of interest. Molecular diagnosis was defined as the identification of a specific genetic change, via analysis of chromosomes (karyotype, chromosomal microarray, FISH), sequencing of a single gene or a panel of multiple genes, deletion/duplication analysis, or methylation studies, interpreted as the cause or probable cause of the patient's clinical presentation. All results of molecular diagnostic tests ordered in the year following the date of the initial Genetics consult were reviewed, and interpretation of findings was verified in clinical notes. ES cases reported by the laboratory as "solved" and "probably solved" were considered diagnosed. For ES patients, other changes in medical management were also tallied through analysis of the clinical note at the time of ES return of results and follow-up in the Genetics clinic.

### ***Aim 3***

Data management and descriptive analyses were performed using Stata/IC 15 (StataCorp, College Station, TX). EMR data was merged with hospital cost data on MRN, and costs were analyzed using the hospital perspective. Costs and



outcomes were evaluated over the time horizon of the index admission and index year.

Establishment of a molecular diagnosis and 1-year survival were the primary outcomes of interest. A comprehensive list of laboratory and radiology diagnostic investigations and associated costs and service dates for each patient over the year following the initial genetics consult order date was obtained from the hospital.

Cost analyses considered costs of the index admission, index admission diagnostic pathway, index admission genetic tests, total cost of the index year, index year diagnostic pathway, and index year genetic tests. The total diagnostic cost included the cost of all activities performed with the goal of making a diagnosis, including ES, targeted genetic testing (single gene or gene panel), inborn error of metabolism screening, MRI, ultrasound, and EEG. Diagnostic cost excluded the cost of other tests performed for non-diagnostic related reasons, such as routine care, through the systematic application of rules for inclusion of the first instance, all instances, or no instances of each type of test. The total cost of the inpatient hospital stay was confined to costs accrued before discharge, even if the patient was discharged before ES results were returned.

Neither costs nor effects were discounted because all costs and outcomes are modeled for one year, so there is no need to account for differences in time preference. All costs were adjusted to 2017 USD\$ using the historical Consumer Price Index for All Urban Consumers (CPI-U): U.S. city average per year.

We account for the skewed distribution of costs by using log transformations and non-parametric statistical tests. We used ordinary least squares (OLS)

regression on log transformed total cost of index admission to estimate the impact of patient characteristics on index admission cost. We employed Wilcoxon rank-sum and Kruskal-Wallis tests for differences in cost categories between cohorts and ES forms, respectively. Chi-square and Wilcoxon rank-sum tests were used for other comparisons as appropriate.

We calculated the cost of the index admission and the diagnostic pathway per percent 1-year survival. We calculated incremental cost-effectiveness ratios (ICERs), the ratio of the difference in expected costs and expected outcomes between one diagnostic strategy and the next most effective is the incremental cost-effectiveness.

Cost-effectiveness of ES versus No-ES is presented in the form of an ICER calculated as:

$$ICER = \frac{\Delta Cost}{\Delta Effectiveness}$$

where

$$\Delta Cost = Cost_{ES} - Cost_{No-ES}$$

and

$$\Delta Effectiveness = Effectiveness_{ES} - Effectiveness_{No-ES}$$

We calculated ICERs for incremental index admission diagnostic pathways costs and incremental diagnoses, and for incremental index admission genetic test costs and incremental diagnoses. For each ICER, 95% confidence intervals were constructed from 1,000 bootstrap replicates.

## **Main Limitations**

The scoping literature review performed for Aim 1 was intended to include all relevant published studies. However, this analysis was limited in that it only included articles that were published in peer-reviewed academic journals. Descriptions of clinical experiences with genomic sequencing published in institutional reports or newsletters would not be detected by the search strategy. Additionally, because the review only considered English language articles, some relevant studies may not be included. However, only one relevant article was unavailable in English.

The analysis performed in Aim 2 and 3 had several limitations. It was a retrospective analysis based on information available in medical records and administrative records, both of which can be incomplete or have other forms of error. Because of the retrospective nature of the study, patients were not randomly assigned to treatment or control group arms. However, this study improved upon previous research by matching patients who received ES to patients who did not on important clinical features in an attempt to control for confounding factors. Although many have called for prospective clinical trials in order to study the patient populations for which cGS is most effective,<sup>90</sup> balancing cohorts by patient phenotype and other relevant clinical characteristics utilized in this study aimed to estimate the impact for specific types of patients while utilizing information available in patient charts.

There were several potential confounding factors that may be important in the study of mortality, especially 1-year survival. This study was limited to data available

in the EMR and ES reports. Estimates of the impact of ES were interpreted based on documentations made in clinical notes, which may have been incomplete. It is possible that the death of some patients may have occurred outside of the hospital and not be documented in the EMR. Similarly, patients may have sought care outside of TCH, in which case costs and encounters would not be documented in the TCH EMR or cost data.

This study used establishment of a molecular diagnosis as an outcome measure, which health economists traditionally consider an intermediate rather than a final health outcome measure. However, receipt of diagnosis is a relevant outcome in this case for both insurers and clinicians. Payers are highly interested in the cost-effectiveness of using these tests to arrive at a diagnosis because it ends the diagnostic odyssey, which involves both diagnostic tests and various specialist visits. For clinicians, a molecular diagnosis can help direct medical management and also provide reassurance about treatment decisions or the decision to discontinue treatment. For both of these reasons, a diagnosis is a useful measure of health outcome in and of itself.

The results of this study cannot be used to draw any conclusions about where in the diagnostic pathway ES should optimally be incorporated (i.e., as a first-line test or subsequent to other tests if they were unsuccessful in establishing a diagnosis). This analysis only looks at pathways with and without ES; it does not attempt to quantify cost-effectiveness for ES at a particular point in the diagnostic pathway (e.g., as a first-line test versus after other diagnostics have been performed).

To address the heterogeneity of the patient population, patients were matched on phenotypic and clinical characteristics. Even so, the underlying cause of disease remained diverse within patient groups because of the number of possible molecular findings associated with any categorization of patients. Unless each molecular diagnosis is modeled separately, which is not practical for sample size reasons, patient groupings will necessarily combine individuals with different genetic etiology. Heterogeneity presents challenges for modelling costs over a longer time horizon than the inpatient stay because each distinct molecular diagnosis will have different prognostic and treatment trajectories. As such, costs and outcomes are only modelled over the time horizon of the index admission and for one year. This allows time for ES to have an impact on care provision and costs, even if results are not returned before discharge from the index admission. As the cost of ES decreases, modelling over a shorter time horizon is more acceptable. Also, from a payer perspective, the shorter time horizon is more relevant than lifetime cost of care projections and may be more informative for coverage determination.

### **Human Subjects Research: Ethical Considerations**

This study involves data that includes protected health information that was collected with patient identifiers. However, because it is a retrospective EMR review study, the only risk to the patient is the possibility of loss of confidentiality. All Excel spreadsheets were password encrypted, and data were stored on Box, the secure online cloud storage and file management service preferred by Baylor College of Medicine. There are potential benefits to future patients based on the findings of this

study, as results may influence patient access to cGS through informing the development of clinical and payer policy. The Baylor College of Medicine Institutional Review Board approved this research. A protocol was submitted for expedited review by The University of Texas Health Science Center (UTHSC) Committee for Protection of Human Subjects and approved.

## JOURNAL ARTICLES

### **Clinical Application of Genome and Exome Sequencing as a Diagnostic Tool for Pediatric Patients: a Scoping Review of the Literature**

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#### **ABSTRACT**

**Purpose:** Availability of clinical genomic sequencing (CGS) has generated questions about the value of whole genome and exome sequencing as a diagnostic tool. Analysis of reported CGS application can inform uptake and direct further research. This scoping literature review aims to synthesize evidence on the clinical and economic impact of CGS.

**Methods:** PubMed, Embase, and Cochrane were searched for peer-reviewed articles published between 2009 and 2017 on diagnostic CGS for infant and pediatric patients. Articles were classified according to sample size and whether economic evaluation was a primary research objective. Data on patient characteristics, clinical setting, and outcomes were extracted and narratively synthesized.

**Results:** Of 171 included articles, 131 were case reports, 40 were aggregate analyses, and 4 had a primary economic evaluation aim. Diagnostic yield was the only consistently reported outcome. Median diagnostic yield in aggregate analyses was 33.2% but varied by broad clinical categories and test type.

**Conclusion:** Reported CGS use has rapidly increased and spans diverse clinical settings and patient phenotypes. Economic evaluations support the cost-saving potential of diagnostic CGS. Multidisciplinary implementation research, including more robust outcome measurement and economic evaluations, are needed to demonstrate clinical utility and cost-effectiveness of CGS.

## **Introduction**

Genome-scale next-generation sequencing (NGS) is increasingly applied in clinical settings as a diagnostic tool, indicative of the arrival of an era of medicine with the capacity to provide patient care guided by genetic makeup.<sup>1</sup> Clinical genomic sequencing (CGS), which includes whole genome sequencing (WGS) and whole exome sequencing (WES), is unique in the realm of diagnostic tests for two primary reasons. First, results of a single test can both establish a molecular diagnosis and inform tailored medical management (i.e., precision medicine) where applicable. Second, the clinical utility of CGS increases with additional application. Uptake influences diagnostic effectiveness because as more patients are sequenced, detected variants are published in case reports and deposited into public databases, which increases the number of known disease genes and in turn impacts future diagnostic performance of the test.



The interplay of these two qualities is important as genetic research is translated into genomic medicine. Since WES became commercially available as a clinical test in 2011, uptake has been sufficient to generate real world evidence on the ability of CGS to provide a molecular diagnosis and impact patient care. Implementation research is suited to explore the context-dependent and dynamic nature of such evidence.<sup>2</sup> In an analytical framework of technology translation, synthesis and analysis of reported findings from initial use in the clinic can inform evidence-based practice guidelines and future clinical application.<sup>3</sup> Both case reports and larger-scale studies of institutional implementation are informative at the current stage of evaluation. Case reports demonstrate the breadth of clinical areas in which CGS has been successfully applied. Studies of larger numbers of patients provide aggregate data on diagnostic yield for different forms of the test (e.g., trio versus proband-only, rapid versus non-rapid), and patient subgroups according to phenotype or clinical setting.

Diagnostic potential of CGS has been seen as particularly powerful for infant and pediatric patients because determination of molecular etiology early in life may enable more timely and specific intervention with a better chance of improving outcomes.<sup>4,5</sup> Infants who are challenging to diagnose by other modalities because of incomplete, atypical, or blended phenotypes stand to benefit from the multiplex nature of CGS because it does not rely on clinical suspicion of the particular gene implicated. Avoidance of sequential single gene or gene panel testing can save time, which is valuable because time to diagnosis can impact the availability or effectiveness of clinical intervention.<sup>6</sup>

Establishment of clinical utility of CGS is a primary concern for clinical implementation and the interdependent development of health care payer policy. Careful evaluations of CGS utilization can inform optimal integration of genome-wide sequencing into diagnostic testing algorithms – where and how to best incorporate CGS into the diagnostic workup for which patients. This involves determining how CGS fits into the landscape of diagnostic decision-making that includes choices between forms of genetic investigation, including targeted genetic tests such as single gene and gene panel tests, complementary tests such as microarrays and copy number analysis, and CGS,<sup>7</sup> which may be performed in addition to or in place of other non-genetic investigations. Although sequencing has typically been recommended for patients with nonspecific clinical features that may be associated with numerous underlying causes (even those which are not yet well established),<sup>7,8</sup> it may be possible to more precisely define types of patients who are the best candidates. Development of such guidelines requires assessment of patients' clinical characteristics and effects of CGS on medical management to determine the types of patients most likely to benefit from CGS and its appropriate position in the sequence of diagnostics.

Value assessment is an important component consistent with precision medicine's goal of choosing the right diagnostic test for the right patient at the right time, especially as costly new diagnostics become available.<sup>9,10</sup> Effectiveness data generated through clinical application studies are required for translational research and are an essential input in economic evaluations to determine the value of the test.<sup>3,11</sup> While numerous methodological challenges exist for economic evaluations

of genomic sequencing tests,<sup>12</sup> measurement of patient health outcomes is perhaps the largest. Difficulty of outcome measurement is not unique to CGS. It exists across all genetic medicine applications, including targeted and disease-specific genetic tests, and contributes to the lack of robust economic evidence on these applications.<sup>13</sup> While diagnostic yield is an important outcome, it is only intermediate measure. More complete assessment of clinical utility would include measures of patients' ultimate health outcome following clinical care provided in light of CGS results.<sup>14,15</sup> Determination of CGS's value for any specific clinically-defined group of patients is further complicated by to statistical uncertainty about outcomes (including diagnostic yield) due to small sample sizes, which can obstruct economic model development.<sup>16</sup>

An understanding of how CGS has been applied in practice, its effects on physician decision-making and clinical care, and how outcomes have been reported is a necessary precursor to full economic evaluation. Technical and cost aspects of NGS compared to the gold standard dideoxy method have been explored.<sup>17</sup> In contrast, evidence on patient outcomes following CGS application has not yet been systematically summarized, which this review seeks to address.

The aim of this scoping review is to provide an overview of published peer-reviewed articles on the application of CGS for diagnostic purposes in infant and pediatric patients. The research questions are: (1) what does the literature say about how diagnostic genome-scale sequencing has been applied in clinical settings for infant and pediatric patients; (2) how have results of these applications been reported; and (3) what was the clinical or economic impact? From studies that report

aggregate-level analyses, information on institutional features, patient population, reported outcome categories, and impact on those outcomes is summarized. From case reports, disease areas and the genetic spectrum in which diagnostic CGS has been applied are synthesized. For studies that aim to estimate the economic impact of CGS, key findings are outlined and the quality of economic evidence reporting is assessed. This review provides an overview of the landscape of CGS since 2009, when proof-of-concept for diagnostic WES was shown.<sup>18,19</sup>

## **Materials and Methods**

### ***Methods***

Scoping reviews are intended to provide an overview of the nature of literature on a topic via structured searches and identify gaps in knowledge. Fewer restrictions for inclusion are placed on patient population, intervention, outcome, and study design than in systematic reviews. This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines,<sup>20</sup> adapted for use in a scoping review as appropriate. CGS is defined to include WGS and WES. Sequencing may have been performed for the proband (i.e., patient) only or alongside parents or other family members (duo or trio), in a non-rapid or a rapid manner with reduced turnaround time. Sequencing was considered clinical rather than research for the purpose of this review if the report's stated goal was to make a diagnosis or otherwise impact medical management of the patient(s). In contrast, if the objective was gene discovery or disease mechanism elucidation, the sequencing was considered research.

A search strategy was designed with the assistance of a librarian from the Texas Medical Center library. PubMed, Embase, and Cochrane Library were searched. The PubMed search included the following Medical Subject Headings (MeSH) terms: Genome; Exome; Sequence Analysis, DNA; Adolescent; Child; Infant; Diagnostic Techniques and Procedures; Clinical Decision-Making; Diagnosis, Differential. Items identified through database searches were imported into the web application Rayyan (Doha, Qatar) for title and abstract screening.<sup>21</sup> Full search strategies are available online as Supplementary Materials and Methods. Two independent reviewers (HSS and SC) screened the title and abstract of each record, and conflicts were resolved through consensus. Citations selected for full-text review were imported into EndNote (Clarivate Analytics, Boston, Massachusetts), and full-text articles were obtained. A full-text review form was completed for each article to determine whether inclusion/exclusion criteria were met. One author (HSS) reviewed each full-text article, and a second reviewer (SC) reviewed a randomly selected 10% of the full-text articles.

Articles that met the following pre-determined criteria were included: (1) peer-reviewed original research article; (2) published between January 2009 and June 2017 (with an updated search performed in November 2017); (3) proband (if a case report) or the majority of probands (if more than 5 probands in study) less than 19 years of age at the time of sequencing; (4) described/evaluated the clinical application of a CGS for diagnostic purposes. Studies of patients who had a clinical diagnosis of a condition with known genetic heterogeneity, and thereby not determined to have a “specific” diagnosis, were included. Studies of patients

enrolled in a research protocol performing CGS for a clinical purpose were included regardless of how costs of sequencing were covered, as the aim of sequencing was considered more important than the funding arrangement. No restrictions were placed on study design; clinical reports (individual cases and case series), intervention studies (any methodology), and economic evaluations (any methodology) were included.

Publications with a primary aim of genetic research were excluded as were publications on population-based screening, tumor genotyping, mitochondrial genome sequencing only (without the nuclear genome), pharmacogenetic testing, disease carrier testing, prenatal genetic testing, and targeted exome sequencing (e.g., “clinical exome” or “Mendeliome”) panels of thousands of genes known to be associated with single-gene disorders. While targeted exomes may be considered more similar to a whole exome than a targeted panel, multiple permutations of such tests exist. Because there is inconsistency in covered genes, publications on targeted tests were excluded for comparability of results and feasibility of this review. Reports on patients who were sequenced post-mortem and those that indicated the initiation of sequencing but not results were also excluded.

Because this scoping review included articles that employed multiple methodologies and studied diverse patient populations, results across studies were summarized and narratively described rather than combined statistically in a meta-analysis. Descriptive statistics were calculated on the number of articles on each type of CGS, characteristics of patients and institutions, clinical scenarios, and reported outcome measures. Discussion of costs and economic evidence was also

summarized. The Consolidated Health Economic Evaluation Reporting Standards (CHEERS) checklist was used to assess the quality of reporting in articles with an economic evaluation focus.<sup>22</sup> Two authors (HSS and HVR) assessed each article independently and arrived at a consensus score.

### ***Data Collection Process***

We developed and pilot tested a data extraction form, and then created two refined versions based on the two types of analyses and reporting encountered. For the purpose of collecting and presenting results in this review, studies of 5 or fewer patients were considered “case reports” and studies of more than five patients were considered “aggregate analyses.” The cutoff number of five was determined based on differences in article structure and information presentation according to the number of patients included. Thus, the data collection form used for each type of study reflected the way in which facts were reported.

Data items selected for abstraction from articles were broadly based on parameters recommended for assessment in evaluation of genetic tests.<sup>23</sup> The data collection form for aggregate analyses included the following items: study objective, country, type of CGS, comparator, clinical setting, study design, outcome measures, study population, inclusion criteria, exclusion criteria, average age at test, percent of probands younger than 19 years of age, percent of probands who were male, diagnostic laboratory, sequencing platform, whether a duo and/or trio approach was used, turnaround time, molecular diagnostic yield, number of probands with a change in medical management, discussion of insurance coverage, discussion of

costs or cost-effectiveness, and average cost to diagnosis or cost of potentially replaced tests. For case reports, the above information was collected on the individual level as well as the gene implicated and diagnosis. For economic studies, the perspective of the analysis, cost data source, and incremental cost per outcome measure were recorded. One author (HSS) abstracted data from all included studies into a spreadsheet. Analysis was performed with Stata IC 13 (College Station, Texas).

## **Results**

### ***Study Selection***

The study selection process is summarized as a PRISMA flow diagram in Figure 1. Database searches and a hand search yielded 3,039 records after duplicates were removed. After review of abstracts, 359 records were selected for full-text review. Following full-text review and resolution of discrepancies by consensus, 135 articles were included and 224 articles were excluded. The inter-rater reliability was high (Cohen's kappa = 0.81) for the 10% of articles receiving a full-text review by two investigators, suggesting good agreement on inclusion/exclusion decisions and unbiased selection of articles for inclusion in this review. The search was updated in November 2017, and an additional 36 articles were included.

### ***Study Characteristics***

Of the 171 total included articles, 131 (76%) were case reports<sup>19,24-153</sup> and 40 (24%) were aggregate analyses.<sup>5,6,154-191</sup> Four studies had a primary objective of



economic evaluation and also reported primary effectiveness data.<sup>153,189-191</sup> The number of included articles increased by publication year. One article each year was included from 2009-2011, 2 from 2012, 7 from 2013, 24 from 2014, 29 from 2015, 48 from 2016, and 58 from 2017. Most studies were conducted in the USA (71) and the European Union (28), followed by Japan (14), Canada (12), China (7), Australia (6) and Korea (5). The first author (or co-first author) listed had a clinical or commercial genetics affiliation for 97 (57%) of articles. Out of 24 items on the CHEERS checklist recommended for reporting, the economic evaluation articles reported 7, 14, 18, and 17 items.

## **Syntheses of Results**

WES was used in 93% (159/171) of articles, WGS in 6% (10/171), and a combination of WES and WGS in 1% (2/171). Of the 98 studies that reported the sequencing platform used, 88% (86/98) were Illumina, 6% (6/98) were Life Technologies, and 3% (3/98) were Thermo Fisher. The majority (22/40) of aggregate analyses reported sequence analysis of proband-parent trios for at least some cases, 5 of which also reported a duo of the proband and mother (or another first-degree relative) in some cases. Turnaround time from test order to result return was reported in 25% (10/40) of aggregate analyses and only 2 case reports. The commercial lab(s) in which sequence analysis was performed was stated in 19 aggregate analyses and 24 case reports, while 16 aggregate analyses and 87 case reports stated that analysis was performed in-house (some of which were College of

American Pathologists-accredited and Clinical Laboratory Improvement Amendments-certified environments).

The 40 aggregate analyses included an average of 225 patients (median = 79; range: 6 – 2,000). Results from the 37 aggregate analyses that did not have a primary aim of economic analysis are summarized in Table 1. Clinical setting and patient population varied widely. Clinical settings included genetics referral centers and hospital specialty clinics (Genetics, Neurology, Epilepsy, Developmental, Dermatology, Mitochondrial Disorders, Hemophilia Treatment), pediatrics departments, and intensive care units. The most common setting was Genetics/Individualized Medicine/Developmental Clinic (12 articles), followed by non-specific children's hospital/university medical center clinic (9 articles) and Pediatric Neurology/Epilepsy/Intellectual Disability Clinic (6 articles). Clinical laboratory (4 articles) and neonatal/pediatric intensive care unit (3 articles) were also reported settings. Most large sample studies (33/37) were retrospective medical record reviews to form a case series (12 of which were sequential) of patients that met specific inclusion criteria for CGS to be performed. All studies that used data from diagnostic laboratories reported information for consecutively obtained samples.

Phenotypic characteristics were used to delineate the types of patients included in each study. All patients lacked a molecular diagnosis at the time CGS was performed by virtue of the inclusion criteria for this review. Phenotype categories were either determined by the study authors, such as organ system affected, severity of disease, or broad phenotypic class (18 articles), or according to

human phenotype ontology (HPO) terms (5 articles). Although the specific category definition varied by study, neurologic phenotypes including intellectual disability (ID)/developmental delay (DD) were a commonly reported phenotypic group (22/37 articles). Diagnostic yield for neurologic phenotypes is presented in Table S1.

Each aggregate analysis reported diagnostic yield, and it was the only consistently reported outcome measure. Where defined, diagnostic criteria were consistent with American College of Medical Genetics and Genomics (ACMG) guidelines.<sup>192</sup> Patients were considered diagnosed if pathogenic or likely pathogenic variant was detected in a disease gene related to phenotype. Diagnostic yield varied by patient population and type of test. Trio sequencing had a higher yield than proband-only when the two were compared (Table 1). Overall diagnostic yield ranged from 8.4 – 100%, with a median of 33.2%. Other than 3 studies that reported 100% yield, the highest yield was 68.3%.<sup>178</sup> Beyond diagnostic yield, other health outcome measures of the downstream effect of sequencing on medical management were listed<sup>5,6,165,173-175,178,180,182</sup> or presented in a table<sup>154,159,172</sup> in 30% of large sample studies. Of the 12 studies that measured them, 8 studies<sup>5,6,172-175,178,182</sup> provided a definition of outcomes, including providing specific examples of the types of care changes included in each category.

Aggregate analyses typically included a summary and discussion of molecular findings, and study authors chose clinically interesting examples to highlight. By nature of the report type, molecular findings dominated the discussion of outcomes in case reports. Table S2 presents implicated genes and the associated diagnoses made in case study patients. Among the case studies, 68% (89/131)

reported a diagnostic finding, 19% (25/131) reported a variant considered by the authors to be the most likely candidate for the patient's clinical presentation, and 9% (11/131) reported a finding that prompted candidate gene association studies. Non-diagnostic findings accompanied by a description of the clinical presentation were reported in 5% (6/131) of case studies. An expansion of the genetic spectrum or clinical phenotype associated with a particular condition was reported in 45% (59/131) case studies.

Overall, 46% (78/171) of articles discussed implications of CGS results on the medical management of patients. Impact on clinical care was more frequently discussed in aggregate analyses (53%, 21/40) than in case reports (44%, 57/131). Likewise, a discussion of economic impact of CGS on the diagnostic workup was more frequently included in larger studies (70%, 28/40) than case reports (15%, 19/131).

Even among the 37 aggregate analyses that did not have a primary objective of economic evaluation, 23 referred to the economic impact of CGS on the diagnostic workup. Several articles specifically stated the need for economic evaluation of such testing (5 articles),<sup>5,6,161,174,185</sup> highlighted that CGS may shorten the time and cost involved in the diagnostic odyssey or sequential single gene testing (6 articles),<sup>5,156,159,162,168,170</sup> or provided an illustrative example or summary statistics on the number or cost of negative diagnostic tests performed prior to CGS (10 articles)<sup>156,162,165,168,171,172,175,182,183,185</sup> which could have been averted if CGS had been utilized as a first-line test. Table S3 summarizes findings from articles that included quantitative results related to economic impact of CGS but that did not have

a primary economic evaluation objective. Only 5 of 37 studies included a comparison group, which was standard diagnostic investigation.<sup>6,162,174,175,183</sup> Insurance coverage of CGS was discussed in 8 large studies and 2 case reports. No formal health state, quality of life, utility values, or specific instruments to measure such outcomes were reported.

Results from economic evaluation studies are presented in Table 2. Each analyzed single-study effectiveness data reported in the same publication. In general, the results suggest that WES can be cost-saving when performed as a first-tier diagnostic test and thus replace serial performance of single gene, gene panel, and other tests. The incremental cost-effectiveness ratio may be considered within acceptable limits even if CGS is employed at later points in the diagnostic trajectory. For example, one prospective analysis in which standard diagnostics were performed in parallel with WES found that first-tier WES was associated with an incremental cost savings of US \$1,702 per additional diagnosis, and when WES was performed after standard diagnostics, the incremental cost per additional diagnosis was US \$6,327.<sup>190</sup> Another study estimated incremental savings of US \$6,840 per diagnosis when WES was performed at the initial tertiary clinical visit and incremental cost of US \$4,371 when WES was used after standard diagnostic investigations.<sup>191</sup> These results underline the role of timing and number of other non-diagnostic investigations performed in whether incremental diagnoses via WES lead to savings or come at an additional cost.

## **Discussion**

In this examination of the published reports of CGS in the pediatric clinical setting, authors of included studies convey enthusiasm about the availability of sequencing technology in the clinic and its potential value as a diagnostic tool. Investigators highlight instances of success in particularly meaningful or puzzling clinical cases. Overall, the results show diagnostic CGS's broad application across clinical settings, increased uptake since commercial availability as measured by the number of publications each year, and high success rates for identification of molecular cause of disease (Table 1). Proliferation of publications appears to reflect diffusion of this diagnostic technology across geographic areas and clinical specialties. Findings of economic evaluations suggest that the multiplex nature of CGS is important for generating value because CGS is capable of replacing other diagnostic tools. However, even if other non-diagnostic investigations are performed prior to CGS, the cost to diagnose an additional patient may still look favorable to decision makers.

Reviewed publications are predominantly retrospective case reports or series across diverse clinical presentations. Among aggregate analyses, 85% employed a retrospective design. Reports to date can largely be classified as descriptive, although quantitative analysis has improved with time and sample size. While there is work to be done to improve the analytical rigor of analyses, particularly in terms of outcome measurement and economic evaluation, this is to be expected in the assessment of a test with paradigm-shifting diagnostic capability. Best practices should be established for measurement and reporting of outcomes subsequent to

sequencing. Standardization would allow more robust analyses to demonstrate clinical utility and cost-effectiveness of CGS. This review suggests multiple candidate categories of outcomes that could be quantified. For example, it may be possible to measure major procedures, imaging studies, or pharmacological intervention averted or initiated as a consequence of GCS results. A framework of standardized category definitions, including specification of procedures and imaging studies considered, and means by which changes are assessed would benefit future research.

Diagnostic yield is the most commonly reported outcome and also the most feasible and straightforward to capture. Results across studies suggest that patient-parent trio sequencing has a higher diagnostic yield than sequencing the proband only (Table 1). Investigators have begun to look at the downstream consequences on patient care; however, categories of clinical impact are not consistently defined or measured. Reported medical management outcomes fall into the following broadly defined categories: surveillance and testing, change in prognosis/impression, subspecialty consult, time to diagnosis, pharmacological intervention, procedure change, imaging change, diet change, palliative care initiation, facility transfer, clinical trial education, family planning, familial genetic testing initiation, genetic counseling, end of diagnostic workup, psychological, and personal/social. Specific wording of outcome categories was not consistent across studies, and details on how assessments were made were rarely provided. Lack of standardization makes comparison across articles difficult. The discussion of care impact in reviewed

articles largely centered on a selected few illustrative cases detailed by study authors.

Follow-up time presents another impediment to outcome measurement. It may not be feasible to ascertain all effects of CGS within the study timeframe. The follow-up period in reported studies was not sufficient to measure potential impacts over the course of the patient's lifetime such as access to school and social programs, disease surveillance, or reproductive decision-making of the proband. Widespread effects of CGS may extend many years after sequencing and to multiple members of the proband's family.

The retrospective nature of the majority of evaluations may introduce selection bias due to preferential reporting and patient inclusion criteria. For each article included in this review, results are specific to the particular clinical population studied. The majority of aggregate analyses employed specific inclusion criteria, sometimes determined by a clinical approval process for CGS specified by the institution. For example, patients may have been required to have already undergone a negative diagnostic workup or meet broadly defined clinical criteria, such as ID/DD, in order to be eligible for CGS. If clinicians selectively include patients whom they have determined CGS would be most likely to yield a diagnosis, the patient sample will not reflect the general patient population. However, the findings will reflect clinical practice and interpretation of results in light of the inclusion criteria may be informative for clinical or institutional policy-making.

There is a risk of publication bias across studies, particularly for case reports and small case series. It is more likely that instances in which CGS was successful



in determining a diagnosis for the patient will be published in a case report. Nevertheless, looking across the clinical spectrum where CGS has been successfully applied can indicate the scope of sequencing as a diagnostic tool. It is possible that some patients reported in case studies may also be included in the cohort of patients reported by the treating institution, where both types of publications exist.

Absence of uniformity in outcome categories and measurement across studies may lead to ascertainment bias, or systematic error based on how a particular researcher defines and records a change in medical management. Similarly, inconsistent methods for costs measurement and medical record data abstraction may impact results of studies that assess costs or the number of previous diagnostic tests performed for each patient. Degree of transparent reporting on cost collection and handling can reveal potential sources of bias, such as how missing data, statistical uncertainty, and currency conversion and indexing were handled. One indicator of this is the quality of reporting as measured by number of items on CHEERS checklist described in the text, which are intended to inform readers about important aspects of how the analysis was conducted. For studies that include an economic analysis, the level of reporting of economic evidence was low, as approximately half of recommended items on the CHEERS checklist were reported on average. Inconsistency impedes comparison across published studies and makes it difficult to draw conclusions. For example, the percentage of patients for whom CGS results affected medical management cannot be directly compared across studies because it depends upon the types of clinical changes considered

and reported in each specific article. At the outcome level, this review is limited by differences in how medical management change is defined by the authors of each study.

Authors of reviewed studies note that the cost-effectiveness of CGS deserves further and more rigorous study and that economic evaluations are an important component of translation to the clinic (Table S3). Discussion of insurance coverage or economics may not have been considered relevant by authors if sequencing was performed under a research protocol. Very few studies have performed a thorough assessment of costs in more than a few example patients. More robust economic evaluation of CGS is needed to quantify the cost effectiveness of testing and to guide reimbursement policy. Of the 4 articles with a primary economic evaluation aim, each limited the cost comparison to the diagnostic odyssey. This may be because outcomes are not clearly defined or because asking what it costs to determine a diagnosis is the most appropriate question at the moment. However, there are numerous cost-related questions that should be explored in future research, such as the cost consequences of earlier diagnosis that may lead to earlier intervention or the decision to not perform medical interventions.

Database searches for this review were limited to PubMed, Embase, and Cochrane. It is possible that additional publications exist outside this search. However, it would be unlikely that relevant studies would not be indexed, and hand searches of other resources supplemented the database searches. This review is limited to articles published in the English language. Inconsistent terminology is a hindrance to systematic searching. WES applied as a clinical diagnostic tool is

sometimes abbreviated clinical exome sequencing (CES) or diagnostic exome sequencing (DES). However, CES is also used to refer to targeted exome sequencing of known disease genes, rather than the entire exome. It was necessary to read details of how the analysis was performed to determine whether it covered the whole exome or only a portion. Additionally, the terms “proband-only” and “singleton” are used interchangeably to refer to sequencing only the patient, and tests with expedited turnaround time are referred to as both “rapid” and “critical.”

## ***Conclusions***

This review is the first to compile evidence on clinical utility of diagnostic CGS for infant and pediatric patients. CGS uptake, as measured by the number of published reports, has substantially and steadily increased since its commercial debut in 2011. It has been applied in a diverse array of clinical settings and demonstrated ability to determine the molecular basis of disease, even in patients who had previously undergone numerous negative diagnostic investigations.

Information on diagnostic yield alone may not be ideal to determine the value of WGS and WES as diagnostic tools. However, downstream outcomes were not consistently defined or reported. While commonly reported information on molecular findings, mode of inheritance, and zygosity are informative for medical geneticists, they do not capture key aspects of CGS relevant for implementation analysis and development of clinical guidelines. Reflecting the dearth of outcomes information, economic analyses have used diagnostic yield as the final health outcome. Lack of standardized outcomes is an obstacle for evaluation of CGS from a health services

research perspective, including determination of cost-effectiveness. Challenges for generating compelling real world evidence of CGS include determination of best practices for defining, measuring, and reporting patient health outcomes subsequent to sequencing. Future studies should aim to reach consensus among experts regarding which outcomes are important and best practices for measurement and reporting. Focus groups or other forms of structured deliberation among stakeholders are potential means to advance this discussion.

As CGS moves toward standard-of-care, more robust evidence of clinical utility and economic and implementation research on CGS are needed. Consistency in outcome assessment is essential for economic analysis input and as part of the technology translation feedback loop. The power of CGS as a diagnostic tool derives from – and must be evaluated within – a dynamic environment that involves both basic science and application in the clinic.

## Tables and Figures

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection

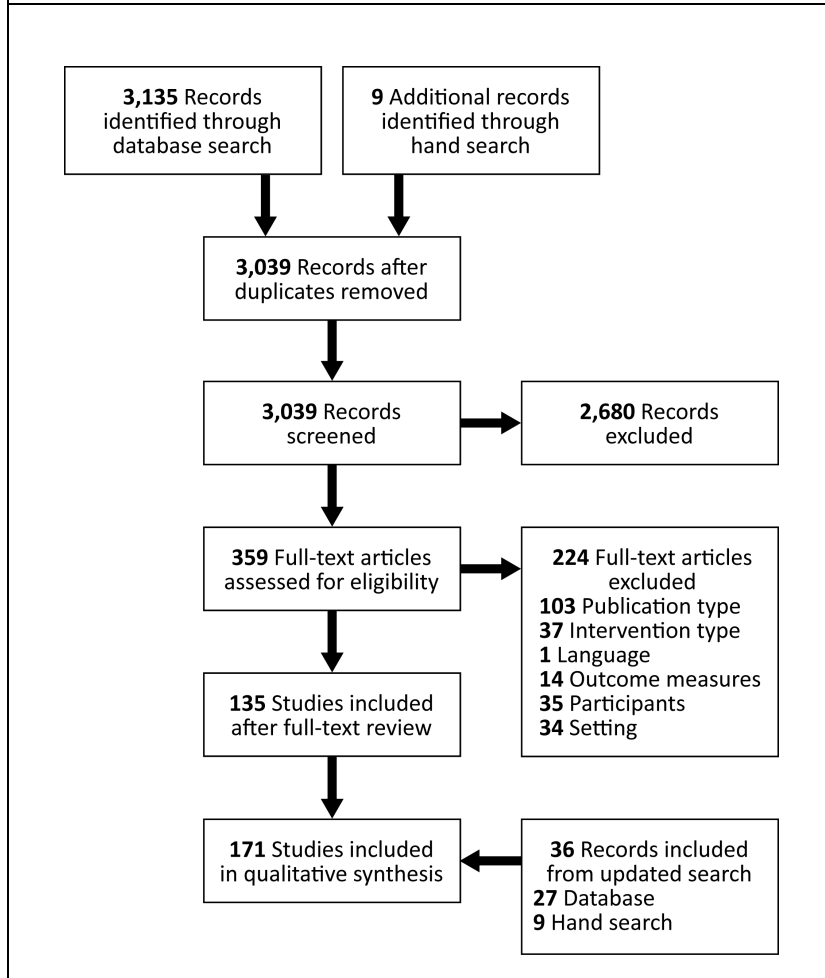


Table 1. Summary of Large Sample Studies				
First Author (Year), Country	Type of CGS	Study Population	Overall Diagnostic Yield (%); Sub-analysis by test type or comparator yield (%)	Change in Mgmt (%) <sup>a</sup>
Bick D (2017), USA <sup>154</sup>	WGS	Suspected Mendelian disorder	3/22 (14); After reanalysis, 8/22 (36)	6/8 (75)
Bowling KM (2017), USA <sup>155</sup>	WES, WGS	DD and/or ID	100/371 (27); Trio: 90/309 (29), Duo: 8/42 (19), Proband: 3/20 (15)	
Farwell KD (2015), USA <sup>156</sup>	WES	Consecutive samples sent to diagnostic lab	152/500 (30); Trio: 82/220 (37), Proband: 14/68 (21)	
Gauthier-Vasserot A (2017), France <sup>157</sup>	WES	Syndromic congenital neutropenia with ID	4/10 (40)	
Helbig KL (2016), USA <sup>158</sup>	WES	Consecutive samples sent to diagnostic lab	322/1131 (28) <sup>b</sup>	
Iglesias A (2014), USA <sup>159</sup>	WES	Consecutive patients in genetics center	37/115 (32)	24/37 (65) <sup>b</sup>
Lazaridis KN (2016), USA <sup>160</sup>	WES	Diagnostic odyssey	15/51 (29)	
Lee H (2014), USA <sup>161</sup>	WES	Consecutive patients referred to clinical lab	213/814 (26); Trio: 127/410 (31), Proband: 74/338 (22)	
Lionel AC (2017),	WGS	Suspected genetic etiology	42/103 (41); Conventional genetic testing: 25/103 (24)	

Canada <sup>162</sup>				
Meng L (2017), USA <sup>5</sup>	WES	Critically ill; suspected monogenetic disorder	102/278 (37); Critical Trio: 32/63 (51), Trio: 13/39 (33), Proband: 57/176 (32)	53/102 (52)
Nambot S (2017), France <sup>163</sup>	WES	Consecutive CA and ID patients	128/416 (31) over 3 years with 2 re-analyses; yield per year ranged 22 – 27%	9/128 (7)
Need AC (2012), USA <sup>164</sup>	WES	ID/DD, CA, or facial dysmorphisms	6/12 (50)	
Nolan D (2015), USA <sup>165</sup>	WES	Neurology clinic	24/50 (48)	8/24 (33) <sup>b</sup>
Ream MA (2014), USA <sup>166</sup>	WES	Drug-resistant epilepsy	1/6 (17)	0/6 (0)
Romasko EJ (2017), USA <sup>167</sup>	WES	Suspected inherited platelet disorder	5/21 (24)	1/5 (20)
Rump P (2016), Netherlands <sup>168</sup>	WES	ID and microcephaly	11/38 (29)	
Sawyer SL (2015), Canada <sup>169</sup>	WES	Diagnostic odyssey	105/362 (29) families	6/105 (6) families
Shamriz O (2017), Israel <sup>170</sup>	WES	Malignant infantile osteopetrosis	6/6 (100)	2/6 (33)
Shashi V (2016),	WES	Outpatient pediatric genetics clinic	24/93 (26) <sup>c</sup>	

USA <sup>171</sup>				
Soden SE (2014), USA <sup>172</sup>	WES, rapid WGS	Neurodevelopmental disorders	53/119 (45) patients, 45/100 (45) families; NICU/PICU: 11/15 (73) families by rapid WGS; Ambulatory: 34/85 (40) families by WES (1 by WGS after negative WES)	22/49 families (45)
Srivastava S (2014), USA <sup>173</sup>	WES	Neurodevelopmental disorders	32/78 (41)	32/32 (100)
Stark Z (2016), Australia <sup>174</sup>	WES	Suspected monogenetic disorder	46/80 (58); Standard diagnostics: 11/80 (14)	15/46 (33)
Stavropoulos DJ (2016), Canada <sup>175</sup>	WGS	Referred for CMA by clinical geneticists	34/100 (34) by WGS; CMA + targeted gene sequencing: 13/100 (13), CMA alone: 8/100 (8)	32/34 (94)
Takeichi T (2013), Kuwait <sup>176</sup>	WES	Pediatric dermatology genetics clinic	7/7 (100)	7/7 (100)
Tammimies K (2015), Canada <sup>177</sup>	WES	Developmental pediatrics clinics	8/95 (8); CMA: 24/258 (9)	
Tarailo-Graovac M (2016), Canada <sup>178</sup>	WES	Potential ID with metabolic phenotype	28/41 (68)	18/41 (44)
Taylor RW (2014), UK <sup>179</sup>	WES	Suspected mitochondrial disease	28/53 (53)	0/28 (0)
Thevenon J (2016),	WES	ID and/or epileptic encephalopathy	14/43 (33); Familial: 6/9 (67)	2/14 (14)



France <sup>180</sup>				
Trujillano D (2017), Germany <sup>181</sup>	WES	Suspected Mendelian disorder	307/1000 (31)	
Valencia CA (2015), USA <sup>182</sup>	WES	Diagnostic odyssey	12/40 (30)	12/12 (100)
Visser LE (2017), Netherlands <sup>183</sup>	WES	Non-acute; neurological symptoms with suspected genetic etiology	44/150 (29); Standard diagnostics: 11/150 (7)	
Willig LK (2015), USA <sup>6</sup>	Rapid WGS	Critically ill; suspected monogenetic disorder	20/35 (57); Standard genetic testing: 3/32 (9)	13/20 (65)
Wortmann SB (2015), Netherlands <sup>184</sup>	WES	Suspected mitochondrial disease	42/109 (39); MD gene "virtual panel": 21/42 (50), outside gene panel (WES): 28/42 (67)	
Yang Y (2013), USA <sup>185</sup>	WES	Consecutive samples sent to diagnostic lab	62/250 (25)	
Yang Y (2014), USA <sup>186</sup>	WES	Consecutive samples sent to diagnostic lab	504/2000 (25)	
Yavarna T (2015), Qatar <sup>187</sup>	WES	Suspected Mendelian disease	89/149 (60)	
Zhang J (2016), Australia <sup>188</sup>	WES	Hematological disorders with suspected genetic etiology	6/6 (100)	

CGS, clinical genomic sequencing; WES, whole exome sequencing; WGS, whole genome sequencing; CMA, chromosomal microarray; ID, intellectual disability; DD, developmental delay; CA, congenital anomaly

<sup>a</sup> Change in medical management overall (any change considered by the study's authors). <sup>b</sup> Author's calculation based on presented data.

<sup>c</sup> According to diagnostic laboratory; clinician interpretation of definite or likely diagnosis in 22/93 (24) patients.

Table 2. Summary of Findings in Economic Evaluation Articles			
First Author (Year) Country/Perspective	Type of economic evaluation; Type of CGS; Comparator	Clinical Setting; Sample size	Cost of Potentially Replaced Tests / Incremental cost per additional Dx by CGS <sup>a</sup>
Joshi (2016) <sup>153</sup> USA / Hospital (not stated)	Descriptive; Trio WES; Standard diagnostics	Epilepsy center; n=4 (including 2 siblings)	Total charges for standard diagnostics range \$9,015 – \$35,483; charge for trio WES \$6,100 / Not Calculated
Monroe (2016) <sup>189</sup> Netherlands / Hospital system	Scenario analysis; Trio WES; Standard diagnostics	Specialty center for intellectual disability; n=17	Average diagnostic odyssey 6.6 years; average cost of traditional diagnostic pathway: \$16,409. For patients who receive Dx, WES to replace genetic tests would save \$4,986 and to replace metabolic tests would save \$2,553, on average. For patients who did not receive Dx, WES to replace genetic tests would save \$5,669 on average. / Not Calculated
Stark (2017) <sup>190</sup> Australia / Hospital system	CEA; Proband WES; Standard diagnostics	NICU, PICU, other inpatient, and outpatient; n=40	Avg. cost per Dx, traditional diagnostics: \$21,099, WES: \$3,937 / WES as a first-tier diagnostic test: savings of \$1,702; WES to replace some diagnostic tests: \$2,045; WES after all other diagnostic tests: \$6,327
Tan (2017) <sup>191</sup>	CEA; Proband	Ambulatory	Avg. diagnostic odyssey 6 years, 19 tests,

Australia / Health care system (not stated)	WES; Standard diagnostics	outpatient clinics; n=44	cost of \$7,509. Cost per patient of WES at initial Genetics appointment \$3,933. / WES at initial tertiary clinical presentation: savings of \$6,840; WES at initial Genetics consult: savings of \$4,143; WES after standard diagnostics: \$4,371
CGS, clinical genomic sequencing; Dx, diagnosis; WES, whole exome sequencing; CEA, cost-effectiveness analysis; NICU, neonatal intensive care unit; PICU, pediatric intensive care unit. <sup>a</sup> All costs reported in USD.			

# Clinical Application of Whole-Genome and Whole-Exome Sequencing as a Diagnostic Tool for Pediatric Patients: a Scoping Review of the Literature

## Supplementary Appendices

### Supplementary Tables

Table S1. Neurologic Phenotype Diagnostic Yield

First Author (Year)	Neurologic phenotype diagnostic yield (%) <sup>a</sup>
Bowling KM (2017)	Intellectual disability: 93/344 (27) <sup>b</sup>
Farwell KD (2015)	Neurologic organ system involvement: 99/324 (31)
Helbig KL (2016)	Epilepsy: 105/314 (33); Non-epilepsy: 212/817 (26)
Iglesias A (2014)	Autism, developmental delay/intellectual disability, neurological/neurodegenerative disorder, and seizures: 11/49 (22) <sup>b</sup>
Lee H (2014)	Global developmental delay in children < 5 years, trio: 45/109 (41); proband-only: 2/23 (9)
Lionel AC (2017)	Referred from Neurology clinic: 3/3 (100)
Meng L (2017)	Abnormality of the nervous system: 42/100 (42)
Nambot S (2017)	Congenital anomaly and intellectual disability: 128/416 (31) over 3 years with 2 re-analyses; yield per year ranged 22 – 27%
Nolan D (2015)	Neurodevelopmental symptoms: 21/53 (40)
Sawyer SL (2015)	Neurodevelopmental phenotype: 31/98 (32)
Soden SE (2014)	Neurodevelopmental disorders: 53/119 (45) children, 45/100 (45) families
Srivastava S (2014)	Neurodevelopmental disorders: 32/78 (41)
Stark Z (2016)	Neurometabolic disorder: 14/19 (74)
Stavropoulos DJ (2016)	Developmental delay: 22/57 (39)

Table S1. Neurologic Phenotype Diagnostic Yield

First Author (Year)	Neurologic phenotype diagnostic yield (%) <sup>a</sup>
Tarailo-Graovac M (2016)	Intellectual developmental disorder and metabolic phenotype: 28/41 (68)
Thevenon J (2016)	Intellectual disability and/or epileptic encephalopathy: 14/43 (33); Familial: 6/9 (67)
Trujillano D (2017)	Abnormality of the nervous system 229/771 (30)
Vissers (2017)	Intellectual disability 78/150 (52); ID with epilepsy or ID with movement disorder 39/150 (26)
Willig LK (2015)	Neurological anomaly 4/7 (57)
Yang Y (2013)	Nonspecific neurologic disorder 20/60 (33); Specific neurologic disorder 4/13 (31); non-neurologic 7/37 (19)
Yang Y (2014)	Neurological 143/526 (27.2); Neurological plus other organ systems 282/1147 (25); Specific neurological 30/83 (36); non-neurological 49/244 (20)

<sup>a</sup> Neurologic diagnostic yield calculated as number of diagnostic cases out of total number of cases with the phenotype

<sup>b</sup> Author's calculation based on presented data

Table S2. Genes and Associated Diagnoses Reported in Case Studies

Year	First Author	Gene(s)	Diagnosis
2017	Aintablian HK	<i>ACAD9</i>	Acyl-CoA Dehydrogenase Family Member 9 (ACAD9) deficiency
2017	Andreoletti G	<i>AMMECR1</i>	Not stated
2015	Arboleda VA	<i>KAT6A</i>	None
2017	Ardicli D	<i>CD59</i>	Inherited CD59 deficiency

Table S2. Genes and Associated Diagnoses Reported in Case Studies

Year	First Author	Gene(s)	Diagnosis
2012	Bacino, CA	<i>WDR35</i>	Sensenbrenner syndrome
2017	Baertling F	<i>VARs2</i>	Valyl-TRNA Synthetase 2 (VARs2) deficiency
2017	Balasubramaniam S	<i>MTP-ATP6</i>	None
2014	Balboa-Beltran E	<i>VEGFC</i>	Milroy-like disease
2014	Bayer DK	<i>IL7R</i>	IL-7R $\alpha$ deficient SCID
2017	Bloom JL	<i>SLC29A3</i>	H syndrome
2017	Bochner R	<i>ABCA12</i> , <i>CAPN12</i>	Likely explanation of clinical phenotype
2017	Boczek NJ	<i>FAM58A</i>	STAR syndrome
2016	Brion M	<i>TAZ</i>	Barth syndrome
2017	Bruel AL	<i>MAB21L1</i>	Likely explanation of clinical phenotype
2016	Çağlayan AO	<i>FTO</i> , <i>CETP</i>	None
2017	Çağlayan AO	<i>ALPK3</i>	<i>ALPK3</i> -associated dilated cardiomyopathy (DCM) that progressed to hypertrophic cardiomyopathy (HCM); Likely explanation of clinical phenotype
2014	Chen M	<i>SBF2</i>	Charcot-Marie-Tooth disease type 4B2 with early onset glaucoma
2017	Chen Q	<i>MMACHC</i>	Cobalamin C (cb1C) deficiency
2015	Chetta M	<i>SMPD1</i>	Niemann-Pick Type A
2016	Chiplunkar S	<i>SLC33A1</i>	Huppke-Brendel syndrome
2016	Chiu ATG	<i>HRAS</i>	Costello syndrome
2009	Choi M	<i>SLC26A3</i>	Congenital chloride diarrhea
2017	Choi R	<i>CPS1</i>	Carbonyl-Phosphate Synthase 1 (CPS1) deficiency

Table S2. Genes and Associated Diagnoses Reported in Case Studies

Year	First Author	Gene(s)	Diagnosis
2015	Choi R	<i>PMM2</i>	Congenital disorder of glycosylation type Ia (CDG-Ia)
2017	Coe RR	<i>CBL</i>	CBL syndrome, or Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia
2014	Das AS	<i>RAPSN</i>	Congenital myasthenic syndrome
2016	Demari J	<i>CLTC</i>	Likely explanation of clinical phenotype
2013	Dhamija R	<i>SCN2A</i>	Likely explanation of clinical phenotype
2013	Dinwiddie DL	<i>IL10RA</i>	Very early onset inflammatory bowel disease (VEO-IBD)
2016	Dionisi-Vici C	<i>GPD1</i>	Glycerol-3-phosphate dehydrogenase 1 (GPD1) deficiency
2014	Dyment DA	<i>ASAH1</i>	SMA-PME
2016	Edvardson S	<i>ACER3</i>	Alkaline ceramidase deficiency (potentially a form of leukodystrophy)
2017	Eskandrani A	<i>AFG3L2</i>	Not stated
2017	Fadus MC	<i>None</i>	None
2014	Fraser JL	<i>TPK</i>	TPK deficiency (Leigh-like encephalopathy)
2015	Gallagher JL	<i>CD40L</i>	X-linked hyper IgM syndrome (XHIGM)
2015	Garg N	<i>AKT2</i>	MORFAN syndrome with hypoinsulinemic hypoglycemia
2017	Gerald B	<i>GNAO1</i>	Early infantile epileptic encephalopathy (EIEE); Likely explanation of clinical phenotype
2015	Goldstein JHR	<i>SMC1A</i>	Cornelia de Lange syndrome (CdLS)
2016	Goodwin G	<i>GATA3</i> , <i>STS</i>	Hypoparathyroidism and hearing loss (HDR syndrome) and X-linked ichthyosis
2016	Guella I	<i>FGF12</i>	Early-onset epileptic encephalopathy (EOEE)
2017	Haberman Y	<i>SI</i>	Congenital sucrase-isomaltase deficiency (CSID)
2015	Harel T	<i>RNF213</i>	Moyamoya disease



Table S2. Genes and Associated Diagnoses Reported in Case Studies

Year	First Author	Gene(s)	Diagnosis
2017	Hasosah MY	<i>NBAS</i>	Infantile liver failure syndrome type 2 (ILFS type 2)
2017	He X	<i>WASP</i>	Wiskott-Aldrich syndrome
2017	Hegde AU	<i>BRAT1</i>	<i>BRAT1</i> -associated epileptic encephalopathy
2017	Hildreth A	<i>NPC1</i>	Niemann-Pick disease type C
2016	Hirabayashi S	<i>MED17</i>	Likely explanation of clinical phenotype
2016	Holzerova E	<i>TXN2</i>	Thioredoxin 2 (TXN2) deficiency; Likely explanation of clinical phenotype
2017	Ichimura T	<i>RPL11</i> , <i>RPS19</i> , <i>RPS7</i>	Diamond-Blackfan anemia (DBA)
2017	Ikeda T	<i>TBCD</i>	Atypical spinal muscular atrophy (SMA) with progressive cerebral atrophy; Likely explanation of clinical phenotype
2016	Inui T	<i>EEF1A2</i>	Epileptic encephalopathy with <i>EEF1A2</i> mutation; Likely explanation of clinical phenotype
2017	Jehee FS	<i>TCF4</i>	Pitt-Hopkins syndrome
2016	Jezela-Stanek A	<i>PGAP2</i> ; <i>PIGN</i>	Inherited glycosylphosphatidylinositol (GPI)-anchor deficiency (IGD); Likely explanation of clinical phenotype
2017	Johannsen J	<i>MTM1</i>	X-linked centronuclear myopathy (CNMX)
2016	Joshi C	<i>PIGA</i>	Phosphatidyl inositol glycan biosynthesis class A protein (PIGA) deficiency
2016	Kansal R	<i>MLH3</i>	None
2013	Keller MD	<i>MTHFD1</i>	Severe combined immunodeficiency (SCID)
2016	Kettwig M	<i>PGAP1</i>	Glycosylphosphatidylinositol (GPI) anchor-related intellectual disability; Likely explanation of clinical

Table S2. Genes and Associated Diagnoses Reported in Case Studies

Year	First Author	Gene(s)	Diagnosis
			phenotype
2015	Khromykh A	<i>HSD17B4</i>	D-bifunctional protein deficiency
2017	Kimizu T	<i>SLC35A2</i>	Early-onset epileptic encephalopathy (EOEE) related to uridine diphosphate (UDP)-galactose deficiency
2015	Kohrogi K	<i>SLC19A3</i>	Biotin-responsive basal ganglia disease
2015	Kuloglu Z	<i>PEPD</i>	Prolidase deficiency (PD)
2016	Kvarnung M	<i>FLVCR2</i>	Fowler syndrome
2016	Lange L	<i>HNRNPK</i>	Kabuki-like syndrome; Likely explanation of clinical phenotype
2015	Law CY	<i>GNAO1</i>	Infantile-onset epilepsy
2017	Leduc MS	<i>HNRNPU</i>	HNRNPU-related disorder; Likely explanation of clinical phenotype
2016	Lee JJY	<i>SIGMAR1</i>	SIGMAR1 deficiency; Likely explanation of clinical phenotype
2015	Lee JS	<i>ATRX</i>	Alpha-thalassemia X-linked intellectual disability (ATRX) syndrome
2016	Lee JS	<i>ST3GAL5</i>	GM3 synthase deficiency
2016	Li N	<i>CYP11B2</i>	Aldosterone synthase deficiency (ASD)
2014	Lim BC	<i>DKC1</i>	Hoyeraal-Hreidarsson syndrome
2017	Lines MA	<i>VAC14</i>	Yunis-Varon syndrome (YVS)
2014	Makrythanasis P	<i>FGFR3</i>	None
2016	Miyamichi D	<i>HPS6</i>	Hermansky-Pudlak syndrome type 6
2016	Mohammad S	<i>PPP1R15B</i>	PPP1R15B deficiency
2015	Mroske C	<i>MTOR</i>	<i>MTOR</i> -related megalencephaly and cognitive impairment;

Table S2. Genes and Associated Diagnoses Reported in Case Studies

Year	First Author	Gene(s)	Diagnosis
			Likely explanation of clinical phenotype
2017	Murray CR	<i>DYRK1A</i> ; <i>KARS</i> ; <i>KAT6A</i>	Developmental and cognitive delay; Likely explanation of clinical phenotype
2017	Nafisinia M	<i>GARS</i>	Mitochondrial respiratory chain dysfunction
2017	Nakamura Y	<i>SZT2</i>	Early-onset epileptic encephalopathy
2016	Naseer MI	<i>STAMBP</i>	Microcephaly-capillary malformation syndrome
2014	Ohashi T	<i>SCN1A</i>	<i>SCN1A</i> -associated epileptic encephalopathy
2017	Ozkinay F	<i>CENPF</i>	Stromme syndrome
2017	Palagano E	<i>FERMT3</i>	<i>FERMT3</i> -associated malignant osteopetrosis; Likely explanation of clinical phenotype
2015	Per H	<i>ABCA1</i>	Tangier disease (TD)
2017	Peragallo JH	<i>AARS2</i>	Alanyl-TRNA synthetase 2 ( <i>AARS2</i> )-related disease; Likely explanation of clinical phenotype
2016	Piekutowska-Abramczuk D	<i>ADAR</i>	Aicardi-Goutieres syndrome type 6 (ASG6)
2011	Pierson TM	<i>AFG3L2</i>	<i>AFG3L2</i> -related spastic ataxia
2016	Pinto AM	<i>CHD2</i>	<i>CHD2</i> -related neurodevelopmental disorder
2014	Pizzino A	<i>TUBB4A</i>	<i>TUBB4A</i> -related hypomyelination
2016	Popp B	<i>GABRA1</i>	Early onset epilepsy; Likely explanation of clinical phenotype
2017	Porntaveetus T	<i>FGFR3</i> ; <i>ALPL</i>	Hypochondroplasia (HCH) and hypophosphatasia (HPP)
2017	Powis Z	<i>RBM10</i>	TARP syndrome

Table S2. Genes and Associated Diagnoses Reported in Case Studies

Year	First Author	Gene(s)	Diagnosis
2014	Priest JR	<i>KCNH2</i>	Long QT syndrome (LQTS)
2015	Prontera P	<i>IGF1R</i>	SHORT syndrome type 2
2015	Punwani D	<i>MALT1</i>	Infantile combined immunodeficiency caused by MALT1 deficiency
2014	Purnell SM	<i>TUBB4A</i>	Hereditary dystonia type 4 (DYT4)
2016	Ramakrishnan KA	<i>MTHFD1</i>	MTHFD1 deficiency
2017	Renkema GH	<i>PET117</i>	Mitochondrial complex IV deficiency
2014	Reuter MS	<i>HIBCH</i>	3-hydroxyisobutyryl-CoA hydrolase (HIBCH) deficiency
2010	Rios J	<i>ABCG5</i>	Sitosterolemia
2016	Sangsin A	<i>COL2A1</i>	Spondyloepiphyseal dysplasia congenita (SEDC)
2016	Santra S	<i>PCK1</i>	Phosphoenolpyruvate carboxykinase (PEPCK) deficiency
2016	Seidahmed MZ	<i>ASNS</i>	Asparagine synthase deficiency (ASNSD)
2014	Shimajima K	<i>TUBA1A</i>	Malfunction of cortical development (MCD)
2015	Shiota M	<i>NRAS</i>	<i>NRAS</i> -related <i>RAS</i> -associated leukoproliferative disease (RALD)
2017	Stanik J	<i>HNF4A</i>	Congenital hyperinsulinism (CHI)
2015	Stiles AR	<i>HIBCH</i>	3-Hydroxyisobutyryl-CoA hydrolase (HIBCH) deficiency
2017	Subramanian VS	<i>SLC5A6</i>	Human sodium-dependent multivitamin transporter (hSMVT) deficiency
2017	Takeda R	<i>GORAB</i>	Geroderma osteodysplastica (GO)
2015	Tamura S	<i>LIG4</i>	DNA ligase 4 (LIG4) syndrome
2015	Thiffault I	<i>POLE1</i>	<i>POLE1</i> -deficiency
2016	Topa A	<i>SBDS</i>	Shwachman–Diamond–Bodian syndrome (SDS)
2017	Tosur M	<i>SLC16A1</i>	Congenital hyperinsulinism; Likely explanation of clinical

Table S2. Genes and Associated Diagnoses Reported in Case Studies

Year	First Author	Gene(s)	Diagnosis
			phenotype
2017	Tsabari R	<i>MYH2</i>	Myosin heavy chain 2 (MYH2) deficiency; Likely explanation of clinical phenotype
2014	Vanderver A	<i>KCNT1</i>	<i>KCNT1</i> -related epilepsy
2016	Vanstone JR	<i>DNM1L</i>	<i>DNM1L</i> -related disease
2016	Varma H	<i>POLG2</i>	<i>POLG2</i> -associated mtDNA depletion syndrome
2017	Villeneuve N	<i>FHF1</i>	<i>FHF1</i> -related early onset epileptic encephalopathy
2016	Wang X	<i>RAF1</i>	<i>RAF1</i> -associated hypertrophic cardiomyopathy (HCM) without Noonan or LEOPARD syndrome
2016	Wasserman H	<i>INS1</i>	Antibody-negative diabetes
2013	Wassner AJ	<i>PROP1</i>	PROP1 deficiency; Likely explanation of clinical phenotype
2015	Wentworth K	GNAS	Likely explanation of clinical phenotype
2017	Wilbur C	<i>ATP1A2</i>	Alternating hemiplegia of childhood (AHC); Likely explanation of clinical phenotype
2016	Williams HJ	<i>PRX</i>	Dejerine-Sottas syndrome
2014	Xia F	<i>AHCD1</i>	Likely explanation of clinical phenotype
2017	Yamamoto T	<i>ASNS</i>	Asparagine synthetase (ASNS) deficiency
2013	Yourshaw M	<i>PCSK1</i>	Prohormone convertase 1/3 (PC1/3) deficiency
2014	Yu HC	<i>KAT6B</i>	Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES)
2017	Zrhidri A	<i>SH3PXD2B</i>	Frank-Ter Haar syndrome (FTHS)

Table S3. Economic Impact Calculations from Large Sample Studies

First Author (Year) Country	Discussion of Economics	Cost of Potentially Replaced Tests
Lazaridis KN (2016) USA	Average cost of WES service \$8,000	Of 43 patients who had other genetic tests prior to WES, 11 (26%) received diagnosis by WES, at an incremental cost per diagnosis of \$27,000 (assuming WES cost \$7,000)
Lionel AC (2017) Canada	Number and cost of negative genetic tests prior to WGS	In 103 total patients, median of 3 conventional genetic tests; median of 19 genes sequenced; CMA in 44 patients; WES in 9 patients; median cost of conventional genetic testing \$5,173 (range \$585 – \$18,361)
Need AC (2012) USA	Cost and reimbursement concerns are a barrier to translation of WES to clinic	One patient had \$22,000 in lab tests prior to diagnosis by WES
Nolan D (2015) USA	Calculate the charge for prior non-diagnostic tests	For patients who had WES, average charge for prior non-diagnostic single gene and gene panels was \$2,465.62
Rump P (2016) Netherlands	WES may be more cost-effective than sequential single gene testing	Average of 2.8 genetic tests (range 0 – 9) performed per family prior to WES
Shashi V (2016) USA	Concerns about cost and insurance coverage can be a barrier to uptake in the clinic	All 93 patients had undergone diagnostic testing (range 1 – 28 tests) prior to WES

Table S3. Economic Impact Calculations from Large Sample Studies

First Author (Year) Country	Discussion of Economics	Cost of Potentially Replaced Tests
Soden SE (2014) USA	Estimate the cost-effectiveness of WES/rapid WGS, as determined by calculating the total cost of prior negative diagnostic tests for patients diagnosed by WES or rapid WGS	For ambulatory care patients, average total charge per family for prior diagnostic testing was \$19,100 (range \$3,248 - \$55,321) for average of 13.3 (range 4 – 36) tests; for NICU/PICU patients, average total charge per family for prior testing was \$9,550 (range \$3,873 – \$14,605) for average of 7 (range 1 – 15) tests. Estimate that WES for ambulatory patients would be "cost-effective," i.e., lower than the cost of non-diagnostic prior tests, at a cost of \$7,640 per family.
Stavropoulos DJ (2016) Canada	Calculate cost of prior non-diagnostic genetic tests for illustrative cases	Average of 3 genetic tests (CMA and two targeted genetic tests) per patient in parallel with WGS; example cases had 3 – 6 tests with a range in total cost of \$3,325 – \$5,280
Valencia CA (2015) USA	Calculate cost of genetic testing prior to WES	19/40 (48%) of patients had at least 4 genetic tests prior to WES, leading authors to conclude "the cost of genetic testing before WES is significant."
Vissers LE (2017) Netherlands	Assessment of WES to replace conventional diagnostic tests	For patients with diagnostic WES, average cost of diagnosis DKK 4,349 (assuming all other genetic tests and standard investigations averted); for patients with non-diagnostic WES, costs would be DKK 10,035 (assuming all other genetic tests averted)
Yang Y	Need future studies of cost-	For one patient, total charges for prior non-diagnostic

Table S3. Economic Impact Calculations from Large Sample Studies

First Author (Year)		
Country	Discussion of Economics	Cost of Potentially Replaced Tests
(2013) USA	effectiveness	genetic testing were 3 times the current cost of WES

## Supplementary Materials and Methods

### Included Studies

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## Complete Database Search Strategies

PubMed Search:

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((((((((((("in patient"[ot] OR "clinic"[ot] OR "clinics"[ot] OR "clinical setting"[ot] OR
office[ot] OR hospital[ot] OR hospitals[ot] OR "NICU"[ot] OR "PICU"[ot])) OR ("in
patient"[tiab] OR "clinic"[tiab] OR "clinics"[tiab] OR "clinical setting"[tiab] OR
office[tiab] OR hospital[tiab] OR hospitals[tiab] OR "NICU"[tiab] OR "PICU"[tiab]))
OR ("Hospitals"[Mesh] OR "Hospital Units"[Mesh] OR "Physicians'
Offices"[Mesh]))) AND (((((((genom*[ot] OR exome[ot] OR "gene panel"[ot] OR
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"next generation sequencing"[ot])) OR ((genom\*[tiab] OR exome[tiab] OR "gene panel"[tiab] OR "next generation sequencing"[tiab])) OR ("Genome"[Mesh:NoExp] OR "Exome"[Mesh] OR "Genome, Mitochondrial"[Mesh] OR "Sequence Analysis, RNA"[Mesh] OR "Sequence Analysis, DNA"[Mesh])) AND (((("Sequence Analysis"[Mesh]) OR (sequence[tiab] OR sequencing[tiab])) OR (sequence[ot] OR sequencing[ot])))) AND (((((neonate[ot] OR neonates[ot] OR child[ot] OR children[ot] OR adolescent[ot] OR adolescents[ot])) OR (neonate[tiab] OR neonates[tiab] OR child[tiab] OR children[tiab] OR adolescent[tiab] OR adolescents[tiab])) OR ("Adolescent"[Mesh] OR "Child"[Mesh] OR "Infant"[Mesh])))) AND (((("Diagnostic Techniques and Procedures"[Mesh] OR "Clinical Decision-Making"[Mesh] OR "Diagnosis, Differential"[Mesh])) OR (diagnosis[tiab] OR diagnostic[tiab] "clinical decision"[tiab] OR "clinical decision"[tiab] OR "medical management"[tiab])) OR ("diagnosis"[ot] OR "diagnostic"[ot] OR "clinical decision"[ot] OR "clinical decision"[ot] OR "medical management"[ot]))))

Embase Search:

('genome'/exp/mj or 'exome'/exp/mj or 'whole exome sequence'/exp/mj or 'whole genome sequence'/exp/mj or 'genome':ab,ti or 'exome':ab,ti or 'whole genome sequence':ab,ti or 'whole exome sequence':ab,ti or 'next generation sequencing'/exp/mj) and ('clinical'/exp/mj or 'diagnosis'/exp/mj or 'diagnostic'/exp/mj or 'differential diagnosis'/exp/mj or 'clinical':ab,ti or 'diagnosis':ab,ti or 'diagnostic':ab,ti) and ('newborn'/exp or 'infant'/exp or 'pediatric'/exp or 'newborn

intensive care'/exp or 'neonatal intensive care unit'/exp or 'pediatric intensive care unit'/exp or 'newborn':ab,ti or 'infant':ab,ti or 'adolescent':ab,ti)

Cochrane Search:

#1 [mh genome]

#2 [mh exome]

#3 “next generation sequencing”

#4 whole genome sequencing

#5 whole exome sequencing

#6 gene panel\*

#7 #1 or #2 or #3 or #5 or #6

#8 clinic

#9 “diagnosis”

#10 “diagnostic”

#11 #8 or #9 or #10

#12 “newborn”

#13 “neonatal”

#14 “pediatric”

#15 #12 or #13 or #14

#7 and #11 and #15



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## **Exome Sequencing for Critically Ill Infants Compared to Standard Diagnostics: A Retrospective Analysis of Clinically Matched Cohorts**

**Target Journal:** *Genetics in Medicine*

### **Abstract**

**Purpose:** To estimate the effectiveness of clinical exome sequencing (ES) for patients with a suspected genetic etiology admitted to intensive care within the first year of life.

**Methods:** We analyze ES application at a large children's hospital over 5 years using electronic medical record data. We examine uptake of ES forms among attending Geneticists. We compare outcomes between cohorts of clinically similar, critically ill newborns and infants with a suspected genetic etiology who had ES (n=368) and diagnostic workup without ES (n=368). Main outcomes are establishment of molecular diagnosis and survival over a one-year time horizon.

**Results:** We found variability in ES ordering practice at the provider level. Molecular diagnostic yield (25.8% No-ES, 27.7% ES;  $p=0.56$ ) and 1-year survival (84.8% No-ES, 80.2% E;  $p=0.10$ ) were similar for patients who had ES and patients who had standard-of-care diagnostic investigations other than ES.

**Conclusion:** As clinically applied, ES is an important diagnostic tool, as are chromosomal microarray and targeted genetic testing, for diagnosing patients with a severe clinical presentation within the first year of life. Further work to define utility of

ES testing not captured by diagnostic yield is warranted to develop clinical guidelines for the appropriate application of ES.

## **Introduction**

Clinical genomic sequencing (cGS) has increased capacity to make robust molecular diagnoses of genetic disorders, even those difficult or impossible to clinically diagnose.<sup>1,2</sup> Exome sequencing (ES) has demonstrated ability to diagnose critically ill newborns and infants influence medical management, especially when results are returned in an expedited fashion.<sup>1-6</sup> Robust evidence of ES clinical utility to aid clinical guideline development, however, is currently sparse but is an active area of investigation.<sup>7-9</sup> Very little is known about the impact of ES compared to standard diagnostics in real-world clinical practice.

Evaluation of patients with suspected genetic disorders is important to pediatric and neonatology practice due to incidence of genetic diseases that manifest at birth or soon after. A leading cause of mortality in infancy, genetic disorders afflict more than one quarter of patients admitted to a level IV neonatal intensive care unit (NICU) who die before age 5.<sup>10,11</sup> Both chromosomal abnormalities and single-gene disorders contribute to this disease burden.<sup>12,13</sup> Diagnostic workup for a suspected genetic condition is associated with longer NICU stay,<sup>11</sup> and diagnoses of genetic-based diseases are associated with longer, more costly pediatric inpatient stays.<sup>14,15</sup>

Most prior studies of ES have been conducted within a research framework, with inclusion criteria such as clinician review and prior negative diagnostic testing, more appropriate to establish efficacy of ES to identify an accurate diagnosis, rather than

effectiveness. Moreover, most studies do not include a comparison group of patients who did not have ES,<sup>7</sup> which prevents conclusions about relative effectiveness in a population.

Identification of an appropriate patient population and measurement of outcomes are both key challenges for generating evidence of cGS effectiveness.<sup>8,16-18</sup>

Selection of a comparator group of patients is necessary to quantify ES impact relative to usual care yet difficult in retrospective analyses and in prospective trials involving critically ill children for whom withholding of sequencing may generate ethical concerns.<sup>19</sup>

Outcome measurement is uniquely challenging in evaluations of cGS. Because ES analyzes changes at a genome-wide scale, it is capable of diagnosing nearly any of the more than 5,000 different single-gene disorders with a known molecular basis,<sup>20</sup> meaning there is no one natural history of disease which can be modeled. Further, there is no standardized position of ES in the diagnostic pathway, as the workup for a suspected genetic disorder is largely left to the discretion of clinical geneticists caring for a particular patient. Diagnostic yield has therefore most widely used as a summary measure of outcome,<sup>7</sup> as many downstream clinical outcomes vary at the disease-level. Because many diseases diagnosed by ES are very rare individually, disease-specific outcomes are sparse.

We aim to evaluate the impact of ES on outcomes compared to standard diagnostics, including other genetic tests, for critically ill newborns and infants with suspected genetic disease. We study uptake and use of ES over more than 5 years at a large children's hospital, reflective of effectiveness as clinically applied. We

employ a historical cohort design to enable comparisons between clinically and phenotypically similar patients who did and did not have ES as part of a diagnostic workup in the first retrospective, comparative analysis of ES in a large patient population. In this paper, we (1) describe a population of infants in intensive care who had a suspected genetic etiology and underwent diagnostic testing; (2) describe uptake of ES in its various test forms; (3) examine health outcomes, including molecular diagnostic yield and survival, in critically ill patients who did and did not have ES in the first year of life.

## **Patients and Methods**

We analyzed ES application at a large children's hospital during the initial uptake period of December 2011 through June 30, 2017. The study population is patients with a suspected genetic etiology who had an intensive care unit admission within the first year of life. Patients were admitted to Texas Children's Hospital (TCH) in Houston, TX, a large children's hospital with 33,000 total admissions in 2017 and the highest level of neonatal intensive care (Level IV).<sup>21</sup> Documentation of an inpatient consult from the Genetics service indicated that the patient was suspected to have a genetic etiology. All TCH ES orders were sent to Baylor Genetics (BG) diagnostic laboratory. Electronic medical record (EMR) review was completed in August 2018 such that there is a minimum of one year of clinical follow-up data on all patients. Data analysis was performed using Stata 15 (StataCorp, College Station, TX).



### ***Identification of Eligible Patients***

We employ a retrospective cohort study design with ES as the exposure factor. Two patient cohorts were defined; patients who had ES as part of a diagnostic workup (ES cohort) were matched, based on clinical characteristics and phenotypic presentation, to patients who did not (No-ES cohort).

Two datasets were combined to define the patient population. The first dataset, obtained from the hospital, contained medical record numbers (MRNs) of all patients at TCH who (1) had an intensive care unit inpatient admission within the first year of life, and (2) had an order for inpatient consultation from the Genetics service. The second dataset, obtained from the diagnostic laboratory, contained ES report data for all patients who had ES (1) ordered from TCH, and (2) an ES order date less than 366 days from date of birth. Datasets were merged on MRN. Patients appearing in both datasets were preliminarily designated as the ES cohort; patients appearing only in the hospital data were preliminarily designated as the No-ES cohort.

EMR review was then performed for all patients. We define the index admission as the admission during which the initial genetics consult was ordered. A consult order and a note from a member of the genetics service constituted consultation. Data on admission characteristics, demographics, phenotypic presentation, clinical outcomes, ES order and result return, and ES uptake by attending clinician was collected from index admission administrative notes and genetics consult and follow-up notes. Ethnicity was recorded as listed in the EMR demographics tab; if unlisted or if information in the demographics tab was

contradictory information to the genetics note, ethnicity was recorded as listed in genetics note, which contained detailed information on family history and country of origin.

Patients had one of 3 forms of ES: sequence analysis of only the patient (proband), a trio of patient and both parents (trio), or trio of patient and parents with expedited turnaround time (critical trio). Clinical ES became available in October 2011 in proband form. The trio test was introduced in October 2014 and critical trio in April 2015. Details of the matching procedure used to select No-ES cohort patients are given in Supplementary Materials and Methods.

### ***Measurement and Comparison of Outcomes***

ES uptake and health outcomes were systematically assessed through EMR review. Because a key assumption in our identification strategy is that clinically similar patients may have had ES or not based on variability in ordering practice at the provider-level, we analyzed the pattern of uptake among different attending Geneticists as different forms of ES (i.e., proband, trio, rapid trio) became available. We extracted the names of signers (trainees) and cosigners (faculty) of the initial Genetics consult note and the Genetics consult note during which ES was ordered.

Establishment of a molecular diagnosis and survival were the primary outcomes of interest. Molecular diagnosis was defined as the identification of a specific genetic change, via analysis of chromosomes (karyotype, chromosomal microarray, FISH), sequencing of a single gene or a panel of multiple genes, deletion/duplication analysis, or methylation studies, interpreted as the cause or

probable cause of the patient's clinical presentation. All results of molecular diagnostic tests ordered in the year following the date of the initial Genetics consult were reviewed, and interpretation of findings was verified in clinical notes. ES cases reported by the laboratory as solved and probably solved were considered diagnosed. For ES patients, other changes in medical management were also tallied through analysis of the clinical note at the time of ES return of results and follow-up in the Genetics clinic. A comprehensive list of diagnostic-related investigations performed for each patient over the year following the initial Genetics consult order date was obtained from the hospital.

We calculated descriptive statistics on demographics of patients and characteristics of the index admission. We produced Kaplan-Meier survival curves to analyze survival time, including survival to 28 days, 1 year, and end of study. We used Cox regression models to analyze survival times and logistic regression models to analyze odds of molecular diagnosis.

## **Results**

A total of 368 patients who had ES comprised the ES cohort, and the 368 patients who comprised the No-ES cohort were selected via the matching process from among 936 patients meeting study criteria who did not have ES (Figure 1). Patients represented a diverse population with home addresses in 361 unique zip codes from across the US.

Patient characteristics were well balanced between cohorts after matching (Table 1). More patients were male, Non-Hispanic, and white. Although race,

ethnicity, preferred language, gestational age at birth, and parental age at birth were not included in the propensity score, they are balanced between cohorts with no significant differences in any of these characteristics. The majority of patients were in the NICU during the initial Genetics consultation, most of which took place within the first quarter of a year of life.

Characteristics of the index admission are presented in Table 2. Most patients were admitted from transfer centers, but admission and Genetics consultation took place soon after birth, a median of 11 and 13 days of life for the No-ES cohort and ES cohort, respectively. Length of stay was longer and distributed differently for ES patients than no-ES patients (Figure 2).

### ***Exome Sequencing Cohort***

Form of ES was proband for 227 (61.7%) patients, trio for 54 (14.7%) patients, and critical trio for 87 patients (23.6%) (Table S2). Median turnaround time was 13 days for critical trio ES and 87 days for the non-rapid versions (95 days for proband and 50 days for trio). Critical trio ES was recommended and ordered sooner after admission (median 9 days) than orders for trio and proband ES (median 25 days,  $p<0.01$ ).

In addition to clinical acuity, availability of biological parents to submit a DNA sample was a factor in form of ES order. Two parents submitted DNA samples in 271 cases (73.6%), one parent in 46 (12.5%), and no parents in 51 (13.86%). In 16 cases (4.3%), the lab received parental samples after the initial ES report and issued an addendum that included interpretation in light of parental sample information. For

the 203 tests performed after critical trio was available, proband still accounted for 31.0% (63/203) of all tests and was ordered in 16.2% (27/167) of cases with a DNA sample able to be obtained from two parents.

ES was ordered at the initial Genetics consult for 205 patients (55.7%), a follow-up Genetics consult during the index admission for 115 (31.3%) patients, during a subsequent inpatient admission for 25 (6.8%) patients, and during a subsequent outpatient clinic visit for 23 (6.3%) patients. Among ES orders entered during the index admission, orders were placed median 24 days before discharge, which is shorter than the median turnaround time for non-rapid tests.

ES resulted before discharge from the index admission for 106 (28.8%) patients. A higher proportion of patients who had critical trio were diagnosed before discharge (24.1%) than patients who had proband or trio tests (5.7%,  $p<0.01$ ).

After 6 months of uptake (June 2012), mean 6 ES tests of any form were ordered per month over the study period (Figure S1). Proband ES orders averaged 4 per month prior to availability of trio forms, after which the average total number of tests ordered per month increased to 7, driven by uptake of trio and critical trio forms ( $p<0.001$ ) as proband orders decreased to 3 per month. There were 19 attending geneticists who rotated on service and authored consult notes over the study period. Total consults, ES order consults, and molecular diagnostic yield by attending geneticist are presented in Figure S2, and ES orders by attending geneticist and form are presented in Figure S3.

### ***Molecular Diagnostic Yield***

Overall, 205 (27.9%) of patients received a molecular diagnosis (Table 3). There was no difference in molecular diagnostic yield between the cohorts. A genetic change determined to be causal or probably causal of the patient's clinical condition was identified in 95 (25.8%) No-ES cohort patients by genetic tests other than ES and 102 (27.7%) ES cohort patients by ES. In addition, 8 patients in the ES cohort were diagnosed by chromosomal microarray (CMA) which are not included in the ES diagnostic yield calculation. Genetic diagnoses and diagnostic tools with which they were identified in the No-ES cohort and ES cohort is given in Table S4 and Table S5, respectively. There was no difference in outcomes within the ES cohort between patients who had different forms of ES (Table S6.) In addition to diagnostic yield, we assessed changes in management in the ES cohort in the following categories: subspecialty referral, medication change, screening recommendation, diet change, redirection of care to comfort care, and surveillance recommendation (Table S7). A Geneticist recommended at least one management change in light of ES results for 81 patients, 49 diagnosed and 32 undiagnosed by ES. In undiagnosed patients, subspecialist referrals and screening recommendations accounted for most management changes, as inconclusive ES results may not definitively rule out the need for clinical monitoring.

Molecular diagnosis odds ratios are presented in Table 4. Overall, Hispanic patients had significantly lower odds of receiving a diagnosis, compared to Non-Hispanic patients, holding other factors constant (model 1,  $p=0.002$ ). In the ES cohort, Hispanic patients had half the odds of diagnosis of Non-Hispanic patients

(model 4,  $p=0.007$ ). The odds of diagnosis for patients in the progressive care unit were approximately 3 times that of patients in the NICU (model 1,  $p=0.004$ ). Patients who were referred for admission by a physician had 2.5 times odds of diagnosis for inborn patients (model 1,  $p=0.009$ ). ES-by-admission year interaction effect was not significant and not included in the final model.

### ***Survival***

A total of 50 (6.79%) patients expired before 28 days of life, 129 (17.53%) before 365 days of life, and 166 (22.55%) before the end of the study period. Among patients who expired, patients in the No-ES cohort expired sooner after birth than ES cohort patients. Table 3 shows 28-day survival of 91.6% of patients in the No-ES cohort and 94.8% in the ES cohort. One-year survival was 84.8% in the No-ES cohort and 80.2% in the ES cohort. At the end of the study period, 80.4% of patients in the No-ES cohort were alive, whereas 74.5% of patients in the ES cohort were alive.

Although there was no difference in age of death between cohorts (Table 3), the pattern of survival shows that a larger proportion of the patients who expired before 28 days of life were in the No-ES cohort, while the proportion that expired at later time points was greater in the ES cohort, which is illustrated by the converging survival curves for the ES and No-ES cohorts (Figure 3). Convergence of the survival curves indicates violation of the proportional hazards assumption and prevents statistical comparison of the cohorts' survival distributions with the log-rank

test. However, inspection of the Kaplan-Meier curve suggests no meaningful difference between the curves.

Survival analysis results from Cox regression models are presented in Table 5. When hazard ratios are calculated within hospital unit, the proportional hazards assumption is satisfied (Table 5, models 4-6). Figure 4 shows Kaplan-Meier survival curves for all patients by unit. Patients who received a diagnosis had 58% higher hazard of death than undiagnosed patients (Table 5, model 4,  $p=0.007$ ). Within the ES cohort, the hazard for diagnosed patients was 92% higher (model 6,  $p=0.004$ ). Figure 5 shows survival curves by cohort and diagnosis category, with lowest 28-day survival in diagnosed No-ES patients and lowest 1-year survival in diagnosed ES patients. Older age at the initial Genetics consultation was associated with a significantly lower hazard rate (Table 5).

## **Discussion**

We estimate the effect of ES for newborns and infants with suspected genetic disease. This is the first analysis, to our knowledge, to compare a group of patients who had ES to phenotypically matched controls in order to do so, and the sample size and comparator group are strengths of the study. Retrospective matching enables us to study the large number of patients who had ES over more than 5 years while also identifying clinically similar patients who received standard-of-care diagnostic workup in order to compare outcomes. Although propensity score matching has been previously suggested as a way to address difficulties in identification of an appropriate comparator group for ES evaluations,<sup>22</sup> we are the



first to apply this approach to identify counterfactual patients and estimate ES effect. Matching on phenotype instead of ultimate diagnosis is advantageous from both a clinical decision-making and study design perspective. The clinical decision point regarding ES order is prior to establishment of a diagnosis, not after, and decisions about diagnostic investigations are based upon the patient's clinical presentation. Moreover, rarity of most conditions diagnosed by ES limits the possible sample size of cases matched on diagnosis.

We find evidence of diagnostic utility of genetic testing overall in newborns and infants with suspected genetic disease, with a molecular etiology identified in 27.9% of patients. We find no difference in diagnostic yield between the cohort of patients who had ES and the cohort of patients who had a standard genetic workup not including ES. These results suggest that CMA and other targeted forms of genetic testing remain important diagnostic tools. CMA has been suggested as the appropriate comparator by which to measure the value of ES.<sup>23</sup> While CMA is first-tier diagnostic standard care, and is therefore an appropriate comparator to evaluate ES against the status quo, ES and CMA detect different types of genetic changes (sequence variants and chromosomal abnormalities, respectively). An astute Geneticist may be able to suspect which type of change a patient has, and therefore the most appropriate test, based on clinical exam.

This analysis examines the effectiveness of ES for patients with suspected genetic disease overall. We do not define requirements for sequential order of testing, diagnostic yield of ES is likely to be higher in the subset of patients with non-diagnostic CMA than we report. While ES is a complementary test to CMA rather

than a substitute for it, genome sequencing (GS) can potentially replace CMA and ES. GS is capable of detecting structural changes and identifying diagnoses not detectable by ES.<sup>24</sup> As such, CMA may be a more appropriate comparator for genome sequencing (GS) than for ES. Further work should be directed toward defining a more rigorous way of categorizing patients according to likely clinical impact of ES to develop clinical guidelines for appropriate use of ES.

Our results highlight several other areas for further investigation. In the ES cohort, Hispanic patients had half the odds of diagnosis of Non-Hispanic patients. This finding speaks to the need for diversity in databases used for variant curation in order to better interpret findings in this group of patients and increase the likelihood of diagnosis.<sup>25</sup> That we find no effect of ES-by-admission year interaction on molecular diagnosis suggests that the rate of molecular diagnosis did not change over time. In other words, neither experiential learning in terms of ES orders on the part of the clinician nor increased genetic knowledge in the field overall appear to have significantly impacted results over our study period.

Our finding of no difference in survival can be interpreted in two ways. First, it may be due to study design and indicate successful matching of patients on severity. Neither cohort was more severely ill, in a broad sense, than the other. Second, it may indicate that ES is not effective at increasing survival compared to standard diagnostics. This finding is not altogether surprising due to rarity of diseases detected by ES for which a treatment may not be available.

Our findings regarding timing of death, however, are somewhat unexpected. No-ES cohort patients expired sooner after birth, yet cGS has been predicted to

increase 28-day mortality driven by redirection to comfort-care only following confirmation of severe, untreatable disease.<sup>26</sup> Our result is better understood in light of the diagnoses in the No-ES cohort, including severe chromosomal abnormalities not compatible with survival, such as trisomy 13, trisomy 18, and other large unbalanced chromosomal rearrangements, that likely drive the higher death rate in the early time period.

The hazard rate for diagnosed patients was significantly higher than that for patients without a molecular diagnosis, driven by results in the ES group. Lower survival for diagnosed patients, compared to patients who did not receive a molecular diagnosis is consistent with other studies of genomic sequencing in the NICU.<sup>9</sup> This indicates the severity of diagnoses identified through ES. Similarly, older age at the initial Genetics consultation was associated with a significantly lower hazard rate; this implies that the most severely ill patients likely to expire within a short time after birth are receiving attention from the Genetics team sooner than patients afflicted with less life-limiting conditions.

We use summary outcome measures of diagnostic yield and survival to describe a heterogeneous population of patients. However, the myriad nuances of outcomes important to determine the effectiveness of ES are not captured in the commonly reported summary measure of diagnostic yield, and this is an active area of exploration. Need for development of a new approach to outcomes measurement and evidence generation for precision medicine applications such as ES has been identified as a top priority by the National Academy of Medicine.<sup>27</sup> Definition and standardization of outcome measures has been recognized by researchers

attempting these evaluations in practice.<sup>28,29</sup> The fact that ES is applicable as a diagnostic tool for theoretically any patient with suspected monogenetic disease, but without prior knowledge of which specific disease or implicated gene, makes it both potentially more effective and more difficult to evaluate at the population level. We have defined boundaries that allow meaningful analysis while preserving the features that make ES unique and useful. Our patient sample is diverse and heterogeneous yet defined by features that stakeholders can use to determine clinical and coverage policy for a distinguishable group of patients.

Real world effectiveness of ES will likely differ by institution. Upon availability as a commercial clinical test, without data on clinical care impact, the technological imperative may have influenced uptake of genomic sequencing of infants.<sup>30</sup> Uptake of genetic testing is influenced by institutional-level factors<sup>31</sup> and other elements which we are not able to systematically study here but should be explored in the future.

We recognize several limitations related to reliance on EMR and administrative data. Objectivity and scalability for a large patient population are advantages of outcome measurement through EMR review, yet potential for incomplete documentation and difficulty in identification of changes in management as a result of ES results are disadvantages. We only measured changes where there was explicit documentation in the note related to the results of ES, whether diagnostic or non-diagnostic, although not all changes may be documented this way. Some ways in which ES results may influence clinical care, such as prognostication,

are especially hard to measure objectively and retrospectively and without the involvement of the treating clinician.

Relatedly, HPO term generation depended upon the information in clinical notes. Clinicians may differ in meticulousness, extent, and quality of phenotypic description, which would, in turn, impact the HPO terms generated from the note. Moreover, HPO terms assessed individually do not capture syndromic patterns of features. Although syndromic features may be caused by both monogenic and chromosomal abnormalities, some patterns may indicate hallmark features of chromosomal abnormalities to clinically indicate CMA instead of ES.

We do not consider non-health outcomes or impacts of ES not documented in the EMR which would require further data collection from patients, families, and clinicians. Empirical data suggest the potential value to families of information, even without possibility of treatment.<sup>32,33</sup> Parents perceive information from ES to have benefits beyond direct clinical usefulness, such as reassurance regarding a transition to comfort care-only measures, help with coping, knowing risk for other family members, and reproductive planning.<sup>18,33-39</sup> Incorporation of family preferences along with objective outcomes can help move toward a more holistic valuation of the cGS and methodological work is needed in this area.<sup>32,40</sup> Moreover, results of cGS may impact clinical management decisions whether or not a definitive diagnosis is established.<sup>41</sup> Valuation of non-health outcomes should be explored for patients, families, and clinicians should be further explored to complement this work.

As clinically applied, ES is an important diagnostic tool, as are chromosomal microarray and targeted genetic testing, for diagnosing patients with a severe clinical

presentation within the first year of life. Combined, nearly 30% of newborns and infants in our study received a genetic explanation of the cause of their clinical features. While this percent may not define the complete spectrum of utility of ES testing, it represents the importance of various diagnostic tools for patients with various genetic disease etiologies.

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## Tables and Figures

Figure 1. Study flow diagram

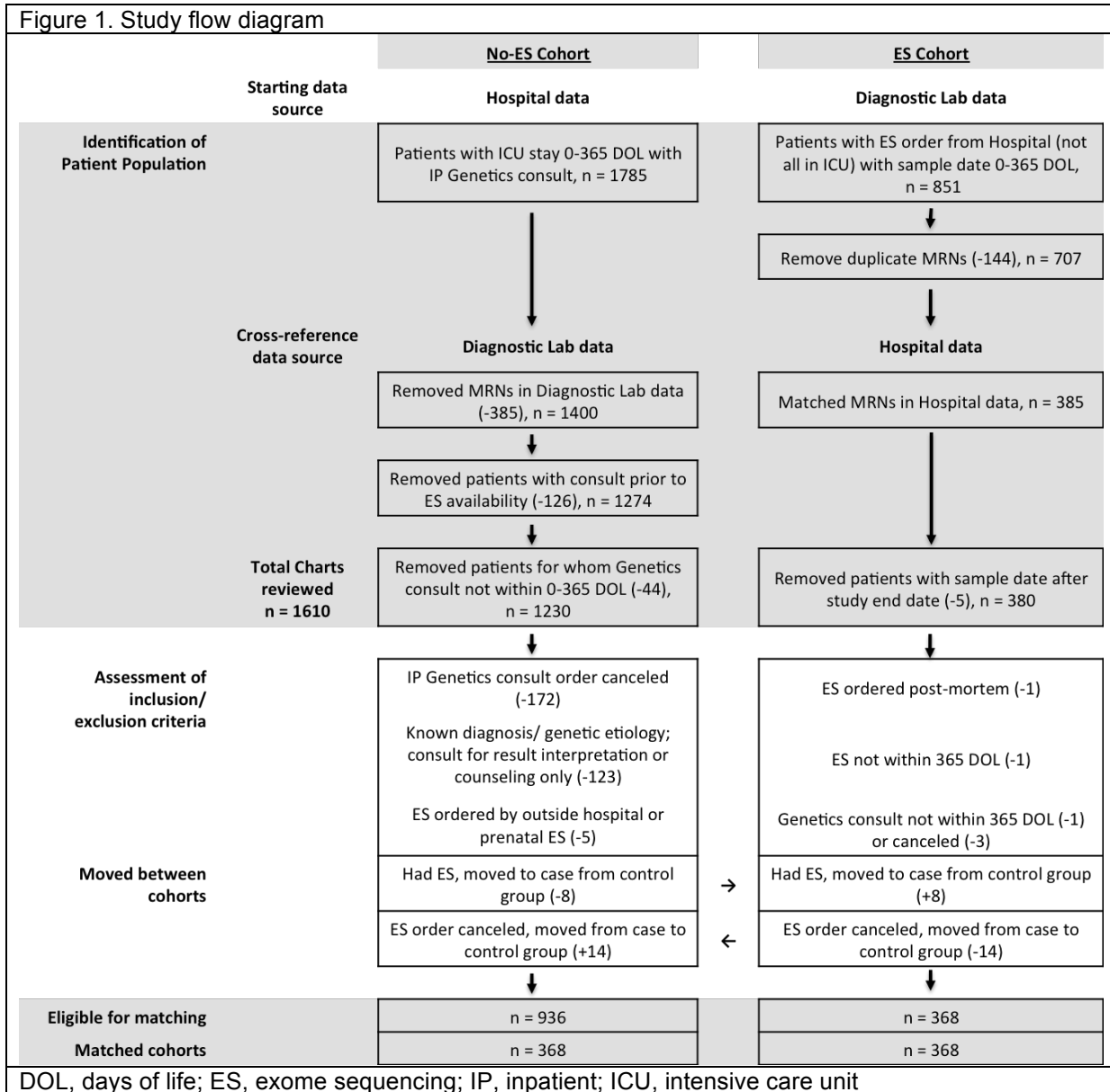


Table 1. Patient Characteristics			
	No-ES Cohort	ES Cohort	p-value <sup>a</sup>
Sex, n (%)			
Male	203 (55.16)	217 (58.97)	
Female	165 (44.84)	151 (41.03)	0.297
Race, n (%)			
White/Caucasian	277 (75.27)	289 (78.53)	
Black/African-American	58 (15.76)	58 (15.76)	
Asian	22 (5.98)	17 (4.62)	
American Indian and Alaska Native	1 (0.27)	1 (0.27)	
Unknown	8 (2.17)	3 (0.82)	0.396
Ethnicity, n (%)			
Non-Hispanic	200 (54.35)	190 (51.63)	
Hispanic	162 (44.02)	174 (47.28)	
Unknown	6 (1.63)	4 (1.09)	0.581
Preferred Language, n (%)			
English	312 (84.78)	294 (79.89)	
Spanish	52 (14.13)	69 (18.75)	
Other	4 (1.09)	5 (1.36)	0.219
Unit of Genetics Consult, n (%)			
NICU	245 (66.58)	222 (60.33)	0.078
CVICU	62 (16.85)	70 (19.02)	0.442

Other	36 (9.78)	36 (9.78)	1.00
PCU	17 (4.62)	25 (6.79)	0.204
PICU	8 (2.17)	15 (4.08)	0.138
Age at Genetics Consult (quartile of year), n (%)			
First	302 (82.07)	297 (80.71)	0.636
Second	43 (11.68)	49 (13.32)	0.504
Third	13 (3.53)	12 (3.26)	0.839
Fourth	10 (2.72)	10 (2.72)	1.00
Genetics Consult Date (quartile of study period), n (%)			
First	92 (25.00)	92 (25.00)	1.00
Second	105 (28.53)	79 (21.47)	0.027
Third	90 (24.46)	95 (25.82)	0.671
Fourth	81 (22.01)	102 (27.72)	0.073
Gestational Age at Birth, weeks, mean (median)	36.40 (37.29)	37.00 (38.00)	0.0711 <sup>b</sup>
Mother's Age at Birth, years, mean (sd)	28.93 (6.27) <sup>d</sup>	28.39 (6.33) <sup>e</sup>	0.251 <sup>c</sup>
Father's Age at Birth, years, mean (sd)	31.60 (7.55) <sup>f</sup>	31 (7.78) <sup>g</sup>	0.357 <sup>c</sup>
ES, exome sequencing; NICU, neonatal intensive care unit; CVICU, cardiovascular intensive care unit; PCU, progressive care unit; PICU, pediatric intensive care unit			
<sup>a</sup> All p-values from chi-square tests unless otherwise noted; <sup>b</sup> Wilcoxon rank-sum test <sup>c</sup> Student's t-test; <sup>c</sup> n = 360; <sup>d</sup> n = 362; <sup>e</sup> n = 312; <sup>f</sup> n = 316			

Table 2. Index Admission Characteristics			
	No-ES Cohort	ES Cohort	p-value <sup>a</sup>
Age at admission, days, median (IQR), mean	1 (0–36), 35.18	2 (0 – 44), 38.30	0.430 <sup>b</sup>
Age at initial genetics consult, days, median (IQR), mean	10.5 (2–54.5), 44.92	13 (2 -57.5), 47.30	0.515 <sup>b</sup>
Point of Origin, n (%)			
Transfer Center	167 (45.38)	160 (43.48)	
Newborn at TCH	128 (34.78)	136 (36.96)	
Self Referral/Non-Health Care Facility	42 (11.41)	40 (10.87)	
Clinic or Physician Referral	31 (8.42)	32 (8.70)	0.928
Length of stay, days, median (IQR), mean	27.5 (10–56), 50.57	39 (17–83.5), 66.86	<0.001 <sup>b</sup>
Discharge Place, n (%)			
Home	294 (79.89)	287 (77.99)	
Expired	51 (13.86)	60 (16.30)	
Other Facility	12 (3.26)	15 (4.08)	
Home Health Care Service	3 (0.82)	3 (0.82)	
Hospice	8 (2.17)	3 (0.82)	0.490
Insurance Payer, n (%)			

Public (Medicaid and Tricare)	194 (52.72)	224 (60.87)	
Commercial	172 (46.74)	142 (38.59)	
None	2 (0.54)	2 (0.54)	0.058 <sup>c</sup>
Second Insurance (public or commercial)	52 (14.13)	67 (18.12)	0.133
ES, exome sequencing; IQR, interquartile range			
<sup>a</sup> All p-values from chi-square tests unless otherwise noted; <sup>b</sup> Wilcoxon rank-sum test; <sup>c</sup> Fisher's exact test			



Figure 2. Index admission length of stay (days) for patients who did and did not have ES

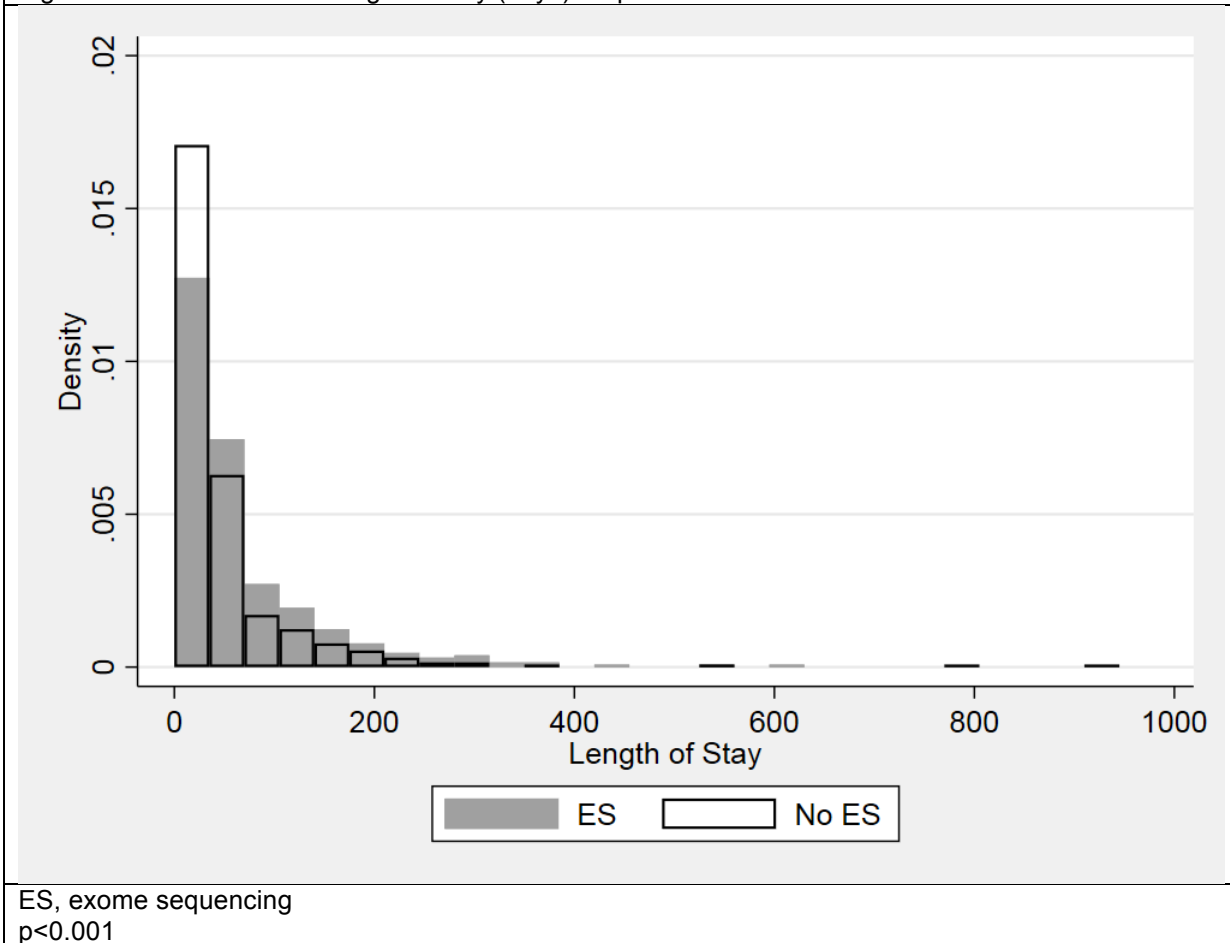


Table 3. Outcomes				
	<b>All Patients (n = 736)</b>	<b>No-ES Cohort (n = 368)</b>	<b>ES Cohort (n = 368)</b>	<b>p- value<sup>a</sup></b>
Diagnostic yield, n (%)	205 (27.85) <sup>b</sup>	95 (25.82)	102 (27.72)	0.560
Survival to 28 days, n (%)	686 (93.21)	337 (91.58)	349 (94.84)	0.079
Survival to 1 year, n (%)	607 (82.47)	312 (84.78)	295 (80.16)	0.099
Alive at end of study period, n (%)	570 (77.45)	296 (80.43)	274 (74.46)	0.052
Age at death, days, median (IQR), mean	69.5 (18–335), 240.90	44.5, (7.5–335), 247.29	121.5 (40–335), 236	0.136 <sup>c</sup>
ES, exome sequencing; IQR, interquartile range				
<sup>a</sup> All p-values from chi-square tests unless otherwise noted				
<sup>b</sup> Additional 8 diagnoses made by chromosomal microarray in ES cohort not included in ES diagnostic yield				
<sup>c</sup> Wilcoxon rank-sum test				

Table 4. Molecular Diagnosis				
	(1)	(2)	(3)	(4)
	All Patients	All Patients	No-ES Cohort	ES Cohort
Molecular Diagnosis	OR	OR	OR	OR
Exome Sequencing	1.145			
	(0.201)			
Female	1.000	0.997	1.183	0.841
	(0.178)	(0.177)	(0.313)	(0.216)
Hispanic	0.576***	0.579***	0.670	0.499***
	(0.103)	(0.104)	(0.175)	(0.129)
DOL Genetics consult	0.998	0.998	0.996*	1.001
	(0.002)	(0.002)	(0.003)	(0.002)
Unit <sup>a</sup>				
CVICU	0.978	0.986	1.574	0.520*
	(0.240)	(0.242)	(0.544)	(0.200)
PICU	2.047	2.108	2.404	1.943
	(0.992)	(1.017)	(2.255)	(1.157)
PCU	2.925***	2.983***	2.717*	3.159**
	(1.091)	(1.111)	(1.639)	(1.546)
Other Unit	1.322	1.333	0.991	1.398
	(0.472)	(0.476)	(0.567)	(0.691)
Point of Origin <sup>b</sup>				
Transfer Center	1.244	1.237	1.028	1.643
	(0.258)	(0.256)	(0.307)	(0.502)

Self Referral	1.217	1.206	0.995	1.495
	(0.404)	(0.400)	(0.473)	(0.724)
Clinic or Physician Referral	2.488***	2.465***	2.329	3.264**
	(0.859)	(0.850)	(1.208)	(1.608)
Admission Year <sup>c **</sup>				
2012	1.186	1.157	0.834	2.444
	(1.171)	(1.136)	(1.253)	(3.458)
2013	0.504	0.499	0.293	1.436
	(0.497)	(0.490)	(0.442)	(2.005)
2014	0.474	0.466	0.276	1.214
	(0.465)	(0.454)	(0.412)	(1.692)
2015	0.662	0.648	0.448	1.271
	(0.647)	(0.630)	(0.667)	(1.766)
2016	0.412	0.406	0.178	1.223
	(0.403)	(0.395)	(0.266)	(1.698)
2017	0.471	0.473	0.139	1.590
	(0.469)	(0.469)	(0.219)	(2.224)
Constant	0.605	0.658	1.119	0.250
	(0.601)	(0.646)	(1.687)	(0.348)
n	726	726	362	364

ES, exome sequencing; OR, odds ratio; DOL, days of life; NICU, neonatal intensive care unit; CVICU, cardiovascular intensive care unit; PCU, progressive care unit; PICU, pediatric intensive care unit

<sup>a</sup> NICU base category; <sup>b</sup> Inborn base category; <sup>c</sup> 2011 base category

\*\*Year variables jointly significant at the 5% level in Model 1

Standard errors in parentheses

\*\*\* p<0.01, \*\* p<0.05, \* p<0.1

Figure 3. Survival by ES cohort

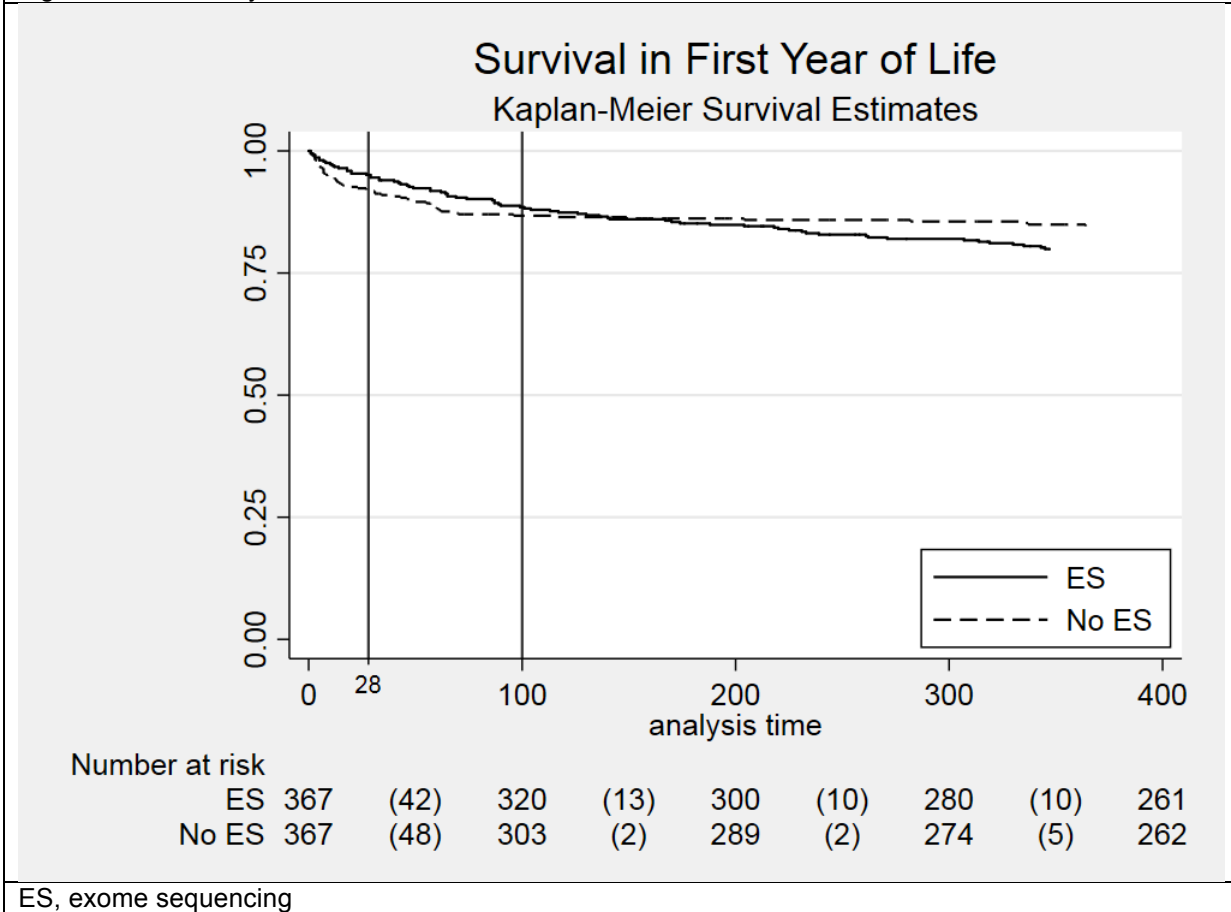


Table 5. Cox proportional hazards regression model						
	(1)	(2)	(3)	(4)	(5)	(6)
Survival	All Patients <sup>a</sup>	All Patients, 28-day Survival	All Patients, 365-day Survival	All Patients, Unit Strata <sup>a</sup>	No-ES Cohort, Unit Strata <sup>a</sup>	ES Cohort, Unit Strata <sup>a</sup>
	HR	HR	HR	HR	HR	HR
Exome Sequencing	1.280	0.608	1.202	1.353*		
	(0.207)	(0.191)	(0.221)	(0.222)		
Molecular Diagnosis	1.535**	1.153	1.371	1.577***	1.186	1.918***
	(0.260)	(0.378)	(0.268)	(0.267)	(0.318)	(0.431)
Female	1.181	1.460	1.101	1.184	1.307	0.957
	(0.189)	(0.440)	(0.201)	(0.192)	(0.333)	(0.207)
Hispanic	1.125	1.420	1.048	1.098	1.342	1.080
	(0.185)	(0.431)	(0.197)	(0.182)	(0.338)	(0.243)
PICU <sup>b</sup>	7.006***	20.304**	7.613***	1.000	1.000	1.000
	(2.141)	(24.296)	(2.506)	(0.000)	(0.000)	(0.000)
CVICU <sup>b</sup>	1.369	1.898*	1.473*	1.000	1.000	1.000
	(0.285)	(0.640)	(0.339)	(0.000)	(0.000)	(0.000)
PCU <sup>b</sup>	1.038	0.000	0.691	1.000	1.000	1.000
	(0.383)	(0.000)	(0.365)	(0.000)	(0.000)	(0.000)
Other Unit <sup>b</sup>	0.696	0.000	0.354*	1.000	1.000	1.000
	(0.265)	(0.000)	(0.217)	(0.000)	(0.000)	(0.000)

DOL Genetics consult	0.997**	0.861***	0.995***	0.996***	0.996*	0.995**
	(0.001)	(0.035)	(0.002)	(0.001)	(0.002)	(0.002)
Medicaid	1.367*	1.242	1.360	1.434**	1.272	1.430
	(0.233)	(0.387)	(0.262)	(0.247)	(0.329)	(0.339)
n	724	724	724	724	361	363
<p>ES, exome sequencing; HR, hazard ratio; CVICU, cardiovascular intensive care unit; PCU, progressive care unit; PICU, pediatric intensive care unit; DOL, days of life</p> <p><sup>a</sup> Survival to end of study period, unless otherwise noted.</p> <p><sup>b</sup> NICU, neonatal intensive care unit base category</p> <p>Standard errors in parentheses</p> <p>*** p&lt;0.01, ** p&lt;0.05, * p&lt;0.1</p>						

Figure 4. Survival by hospital unit, all patients

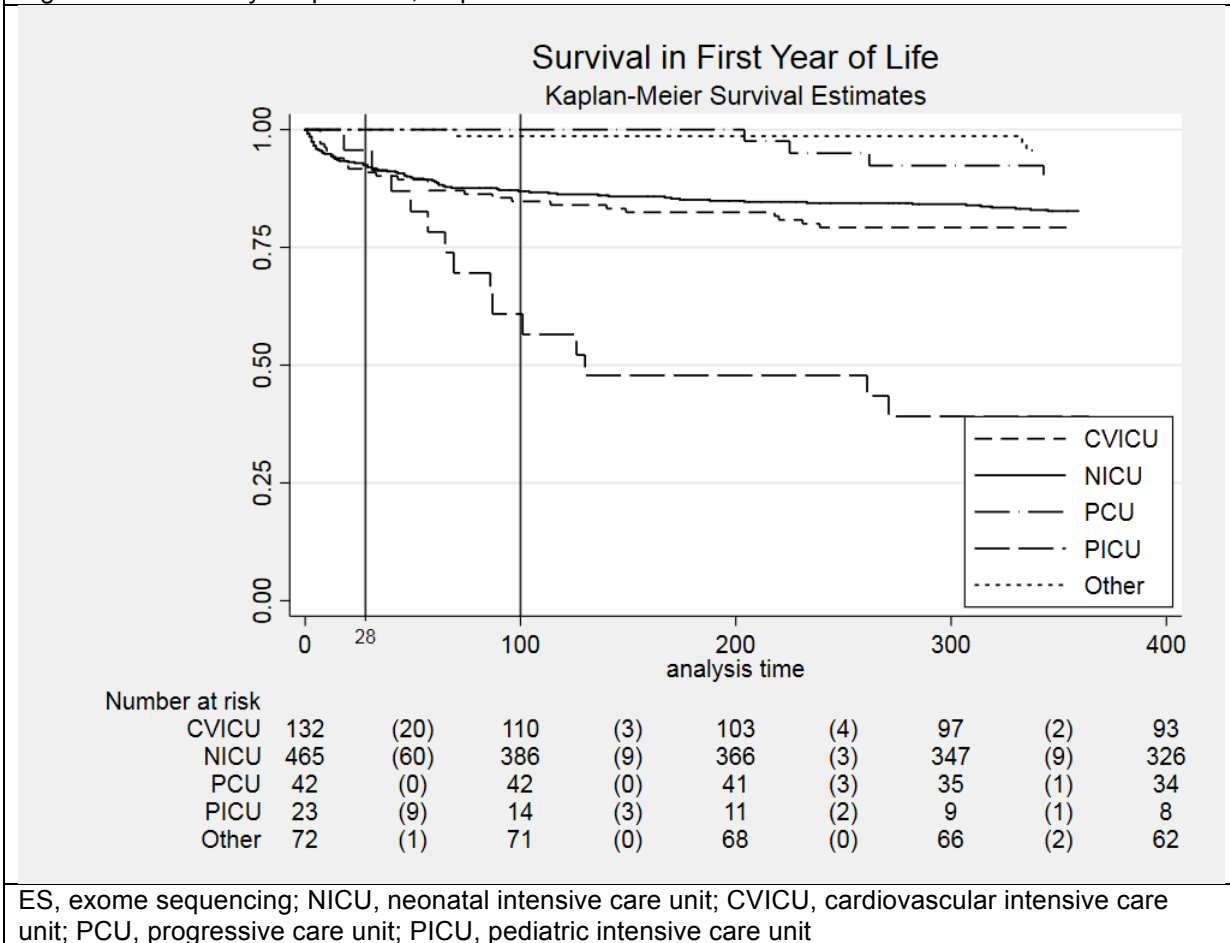
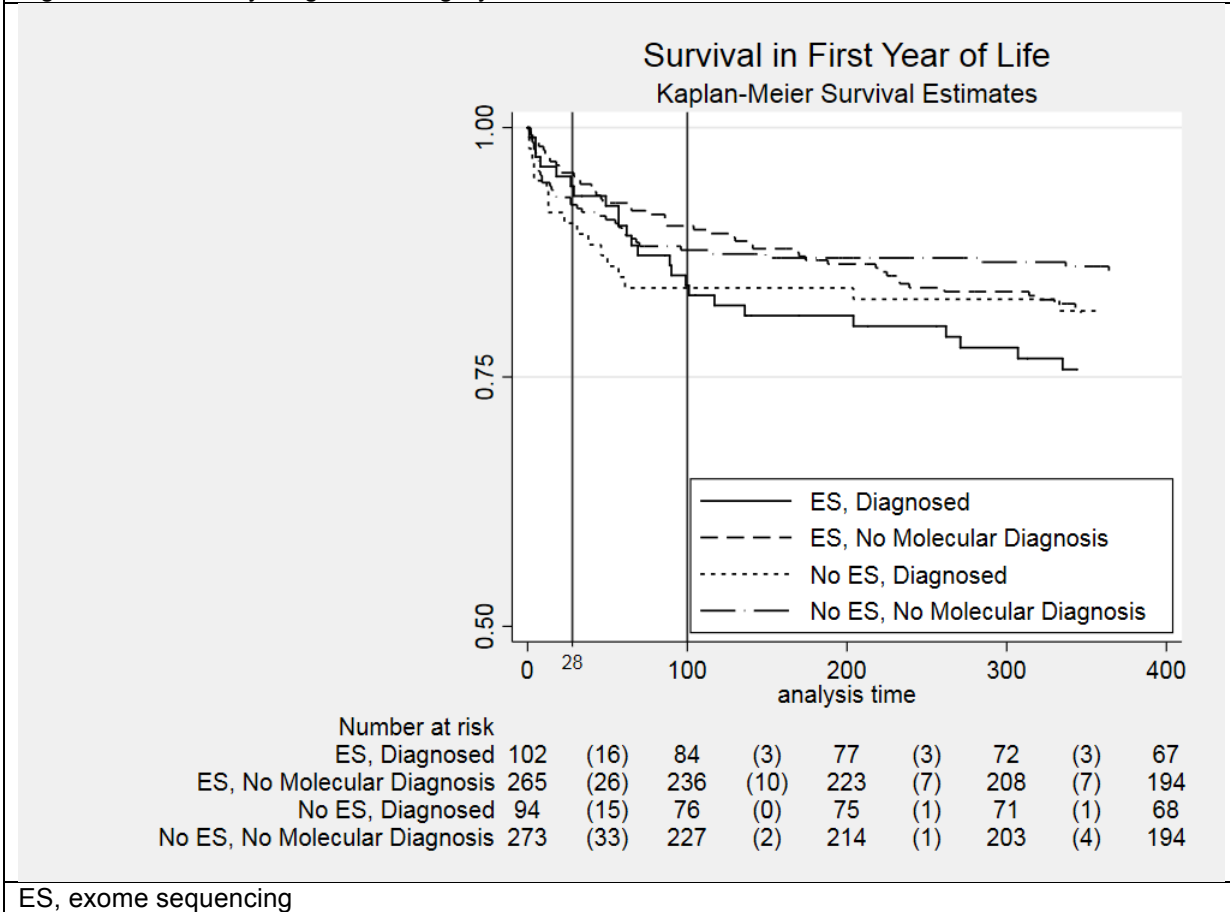




Figure 5. Survival by diagnosis category and cohort



# **Exome Sequencing for Critically Ill Infants Compared to Standard Diagnostics: A Retrospective Analysis of Clinically Matched Cohorts**

## **Supplementary Appendices**

### **Supplementary Materials and Methods**

#### ***Phenotypic Characterization of Patients***

To generate standardized information on phenotypic characteristics across patients, we extracted details about clinical presentation from the initial Genetics consult note (which contains the most extensive and detailed assessment of the patient's clinical features) and index admission discharge note. Relevant information was entered into a natural language processing application that generated a set of human phenotype ontology (HPO) terms for each patient, intended to capture clinical presentation at the time of the Genetics team's assessment. Careful attention was given to avoid inclusion of "pertinent negatives," information from birth or family history, or phenotypic hallmarks of differential diagnoses contained within the note, and the HPO terms for each patient were curated by hand for accurate representation of the note.

#### ***Selection of Matched Controls***

To determine comparable cohorts of patients who did and did not have ES, we calculated a propensity score for each patient. This method allows adjustment for confounding when assessing multiple outcomes.<sup>42,43</sup> The propensity score was used

to represent multiple HPO terms and other relevant factors as a one-dimensional score.<sup>44</sup> We generated a binary variable for each HPO identification (ID) number appearing in the data. HPO ID numbers were used instead of terms themselves to ensure synonymous terms did not appear as separate variables. Granularity was preserved; we did not use hierarchical processing to map to higher order terms (although in many cases, multiple levels of terms were generated). HPO term variables with count fewer than 10, meaning that the term was observed in fewer than 10 patients, were dropped, leaving 340 term variables. Based on clinician consensus, another 33 term variables were dropped because they related to transient clinical characteristics, such as fever or emesis, not relevant for making a diagnosis. HPO terms were selected for inclusion in the propensity score model using a backward automated variable selection process ( $p$ -value for removal = 0.1).

We calculated a propensity score, which is the estimated probability of having ES conditional on measured covariates, for each patient using a binary logistic regression model with ES as the dependent variable that included indicator variables for: gender, the unit in which the initial Genetics consult was performed, initial Genetics consult date (quartile of study period), age (days) at first genetics consult (quarter of year), and HPO terms. After predicting propensity score for each patient, each ES patient was matched to one most phenotypically similar No-ES patient from among all potential No-ES patients using a greedy matching algorithm based on the linear predictor of the propensity score. Compared to differences between ES patients and the entire group of No-ES patients prior to matching, the matching

procedure successfully reduced differences in covariates between ES and No-ES patients in the final cohorts.

## Supplementary Tables and Figures

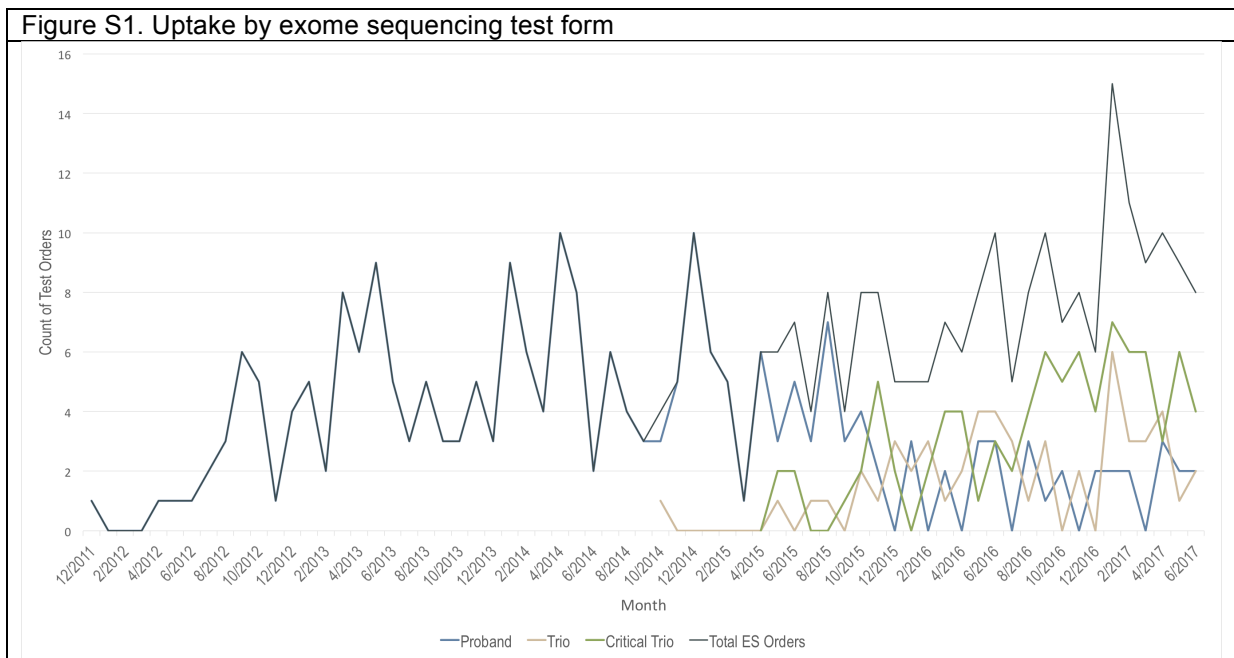
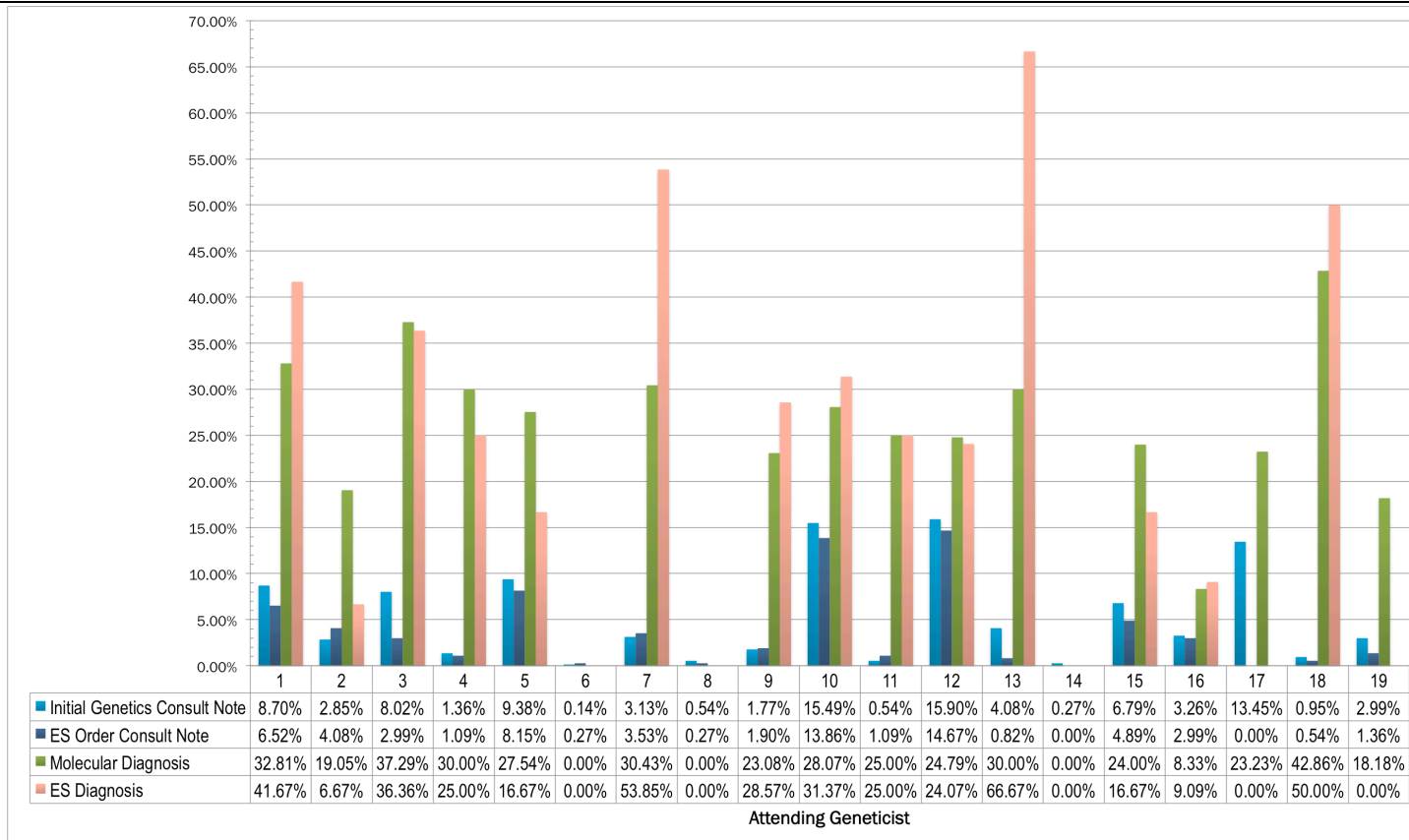


Figure S2. Consults and diagnostic yield by attending geneticist



Initial genetics consult note: percent of initial genetics consult notes among included patients listed as author

ES order consult note: percent of exome sequencing (ES) order consult notes among ES order consult notes listed as author

Molecular diagnosis: percent of patients for whom performed initial genetics consult that received molecular diagnosis by genetic test other than ES

ES diagnosis: percent of patients for whom ES consult note author who received a diagnosis by ES

Figure S3. Form of exome sequencing orders by attending geneticist

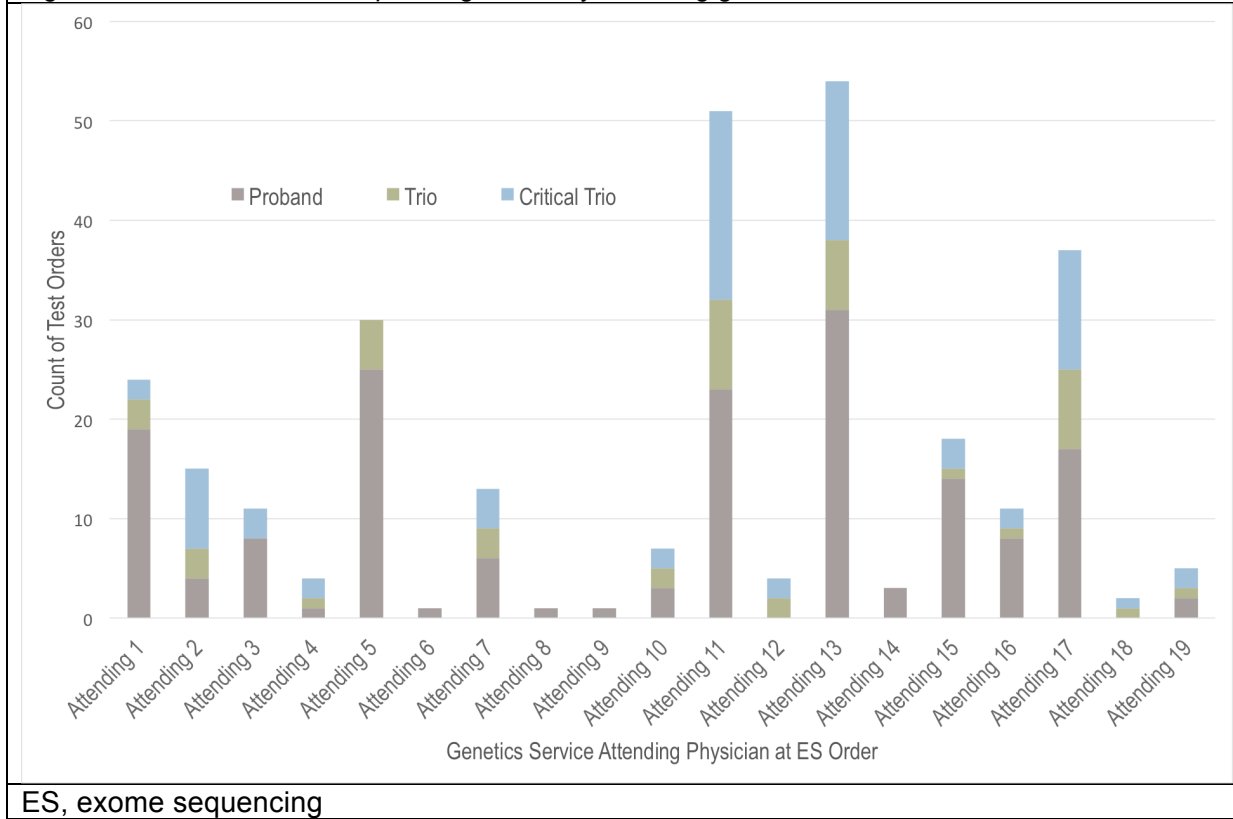


Table S4. Genetic diagnoses and the diagnostic tool with which they were identified in the  
No-ES cohort

	Frequency	Percent
<b>Molecular Diagnosis<sup>a</sup></b>		
17q25.3 deletion	1	1.05
1p36 deletion	1	1.05
30 Mb duplication seen on 15q11.1q21.2	1	1.05
6q deletion	1	1.05
Aicardi-Goutierres syndrome	1	1.05
Apert syndrome	1	1.05
Autosomal recessive polycystic kidney disease	1	1.05
Biotinidase deficiency	1	1.05
Cat eye syndrome	2	2.11
CHARGE syndrome	4	4.21
Chromosome 1 duplication	1	1.05
Chromosome 1 unbalanced rearrangement	1	1.05
Classic Galactosemia	1	1.05
Cobalamin C deficiency	1	1.05
Complex rearrangement of chromosome 8p	1	1.05
Congenital adrenal hyperplasia	1	1.05
Congenital Central Hypoventilation Syndrome (CCHS)	1	1.05
Congenital Tufting Enteropathy	1	1.05
Copy number gain of chromosome bands 18q11.1 to q11.2	1	1.05
Copy number loss of chromosome band 6q26q27 of	1	1.05

approximately 6.907 Mb		
Cornelia de Lange syndrome	1	1.05
Cystic fibrosis	1	1.05
De novo 2.4 megabase duplication on chromosome 13q14.11	1	1.05
De novo 52 Mb gain on 3q24q29 due to unbalanced 3q;15p translocation	1	1.05
De novo copy number LOSS within chromosome bands 19q13.42q13.43	1	1.05
De novo gain of chromosome band 5q23.1q23.2 spanning approximately 1.714 Mb	1	1.05
De novo loss on 4q21	1	1.05
DiGeorge/Velocardiofacial syndrome	8	8.42
DMD duplication	1	1.05
Gaucher disease	1	1.05
Hereditary folate malabsorption	1	1.05
Kleefstra syndrome	1	1.05
Large 11Mb deletion of chromosome 1q4.42.34	1	1.05
LOSS of 5q15q23.2 involving the APC gene	1	1.05
Loss on chromosome 17q12	1	1.05
Maternally inherited UPD 14	1	1.05
Microvillus inclusion disease	1	1.05
Miller-Dieker syndrome	1	1.05
Mosaic Trisomy 8	1	1.05
Mosaicism for partial trisomy/tetrasomy of distal chromosome13q	1	1.05



(13q32.3q34)		
Mosaicism for trisomy 18	1	1.05
Multiple congenital anomalies-hypotonia-seizures syndrome (MCAHS1)	1	1.05
Multiple copy number GAINS within chromosome band Xq28, suggestive of a complex rearrangement	1	1.05
Mutation seen in individuals with the clinical diagnosis of Pfeiffer syndrome (severe), Crouzon syndrome (severe), and Antley-Bixler syndrome	1	1.05
Neurofibromatosis-Noonan syndrome	1	1.05
Noonan Syndrome	2	2.11
Pallister-Killian syndrome	1	1.05
Partial trisomy 18 (~35 Mb)	1	1.05
Rhizomelic condrodysplasia punctata (RCDP)	1	1.05
Russell Silver syndrome	1	1.05
Spinal muscular atrophy	2	2.11
Stickler syndrome	2	2.11
Tetrasomy/AOH of chromosome 9	1	1.05
Duplication of part of CALM2	1	1.05
Trisomy 13	4	4.21
Trisomy 18	3	3.16
Trisomy 21	12	12.63
Trisomy 22	2	2.11
Turner syndrome	1	1.05

Unbalanced translocation	2	2.11
Walker-Warburg syndrome	1	1.05
Williams syndrome	1	1.05
Williams syndrome spectrum	1	1.05
Total	95	100
<b>Molecular Diagnostic Tool</b>		
CMA/FISH/karyotype	68	71.58
Gene panel	9	9.47
Single gene sequencing; deletion/duplication analysis	18	18.95
Total	95	100
ES, exome sequencing; CMA, chromosomal microarray; FISH, fluorescence in situ hybridization		
<sup>a</sup> solved or probably solved/causal or probably causal		

Table S5. Genetic diagnoses and the diagnostic tool with which they were identified in the ES cohort

	Frequency	Percent
<b>Molecular Diagnosis by ES<sup>a</sup></b>		
ABL1-associated syndrome characterized by congenital heart defects and skeletal malformations	1	0.9
Aromatic L-amino acid decarboxylase deficiency (AADCD)	1	0.9
arthrogryposis, renal dysfunction and cholestasis syndrome 1 (ARCS1)	1	0.9
autosomal dominant profound neonatal hypotonia, seizures and encephalopathy	1	0.9
Bardet-Biedl syndrome 1 (BBS1)	1	0.9
cardiomyopathy, dilated, 1FF	1	0.9
cardiomyopathy, familial hypertrophic 2 (CMH2)	1	0.9
CFAP52-related disorder	1	0.9
CHARGE syndrome	2	1.8
Coffin-Siris syndrome 4	1	0.9
COL12A1 related disorder	1	0.9
Combined oxidative phosphorylation deficiency 12 (COXPD12)	1	0.9
congenital disorder of glycosylation	1	0.9
Costello syndrome	1	0.9
Culler-Jones Syndrome (GLI2)	1	0.9
D-bifunctional protein deficiency	1	0.9
de novo likely pathogenic variant in SHANK3	1	0.9

de novo missense variant in CTCF	1	0.9
de novo novel variant in BCAP31	1	0.9
Denys-Drash syndrome	1	0.9
epileptic encephalopathy early infantile	4	3.6
Familial Hemophagocytic Lymphohistiocytosis type 2	1	0.9
Fanconi anemia	1	0.9
Fanconi anemia complementation group D1 (FANCD1)	1	0.9
Gaucher disease type 2	1	0.9
generalized arterial calcification of infancy	1	0.9
Glycogen storage disease type Ia	1	0.9
glycogen storage disease type IV (GSD IV)	1	0.9
granulomatous disease, chronic, X-linked (CGD)	1	0.9
hepatic venoocclusive disease with immunodeficiency (VODI)	1	0.9
insulin-like growth factor 1 resistance (IGF1RES)	1	0.9
intellectual developmental disorder with dysmorphic facies, seizures, and distal limb anomalies	1	0.9
Jeune syndrome	1	0.9
Joubert syndrome	2	1.8
Kabuki syndrome	5	4.5
LAS1L-related intellectual disability	1	0.9
left ventricular noncompaction	1	0.9
lipoyltransferase 1 deficiency	1	0.9
Lowe syndrome	1	0.9
malignant migrating partial seizures of infancy	1	0.9

mandibulofacial dysostosis with microcephaly	1	0.9
Marshall syndrome (MRSHS)	1	0.9
MECP2 related disorder	2	1.8
Megalencephaly-capillary malformation syndrome (MCAP)	1	0.9
mental retardation, autosomal dominant 31	1	0.9
mental retardation, X-linked 9	1	0.9
microcephaly 5, primary, autosomal recessive (MCPH5)	1	0.9
Microcephaly with Pontine and Cerebellar Hypoplasia (MICPCH)	1	0.9
muscular dystrophy-dystroglycanopathy congenital with mental retardation B1 (MDDGB1)	1	0.9
myopathy, centronuclear, 1 (CNM1)	2	1.8
nemaline myopathy	2	1.8
nephronophthisis 3 (NPHP3)	1	0.9
Neurofibromatosis Type 1 and acamptomelic camptomelic dysplasia	1	0.9
Noonan syndrome	9	8.11
novel, de novo BICD2 VUS	1	0.9
OFD1 mutation	1	0.9
Ornithine transcarbamylase (OTC) deficiency	1	0.9
Orofaciodigital syndrome	1	0.9
pancreatic agenesis and congenital heart defects (PACHD)	1	0.9
parietal foramina 2 with modulating effects from TWIST1 variant	1	0.9
Pfeiffer syndrome (PS) type 1	1	0.9

Pompe disease	3	2.7
pyridoxine-dependent epilepsy (PDE)	1	0.9
pyruvate decarboxylase E1 component deficiency (PDHE1 deficiency)	2	1.8
RARS-related leukodystrophy	1	0.9
renal tubular dysgenesis (RTD)	1	0.9
restrictive dermopathy	1	0.9
Rett syndrome	1	0.9
SCN1A-related seizure disorder	1	0.9
short-rib thoracic dysplasia 3 with or without polydactyly (SRTD3)	1	0.9
Smith-Kingsmore syndrome (SKS)	1	0.9
split-hand/foot malformation 6 (SHFM6)	1	0.9
titinopathy	1	0.9
Townes-Brocks syndrome	1	0.9
transient infantile liver failure (LFIT)	1	0.9
transposition of the great arteries dextro-looped 3 (DTGA3)	1	0.9
TRMU-associated transient infantile liver failure	1	0.9
TTN compound heterozygous variants	1	0.9
VUS in gene associated with lissencephaly 4 (with microcephaly)	1	0.9
Walker-Warburg syndrome	1	0.9

<b>Molecular Diagnosis by CMA</b>		
solved by CMA; 10 Mb loss in 15q11q13	1	0.9
solved by CMA; 5.9 Mb deletion in chromosome 2q37.2q37.3	1	0.9
solved by CMA; de novo, loss of 2p14p13.3 (B<P10)	1	0.9
solved by CMA; loss 4q34.1q35.2	1	0.9
solved by CMA; Trisomy 21	2	1.8
solved by CMA; unbalanced translocation, 46,XY, der(6)t(X;6)(q26;q27)	1	0.9
solved by CMA; Xp22.31p22.33 loss	1	0.9
Total	111	100
ES, exome sequencing; CMA, chromosomal microarray; FISH, fluorescence in situ hybridization		
<sup>a</sup> solved or probably solved/causal or probably causal		

**Table S6. Outcomes by exome sequencing test form**

	<b>Proband ES (n = 227)</b>	<b>Trio ES (n = 54)</b>	<b>Critical Trio ES (n = 87)</b>	<b>p- value<sup>a</sup></b>
Diagnostic yield, n (%)	61 (26.87)	13 (24.07)	28 (32.18)	0.523
Survival to 28 days, n (%)	213 (93.83)	52 (96.30)	84 (96.55)	0.544
Survival to 1 year, n (%)	182 (80.18)	45 (83.33)	68 (78.16)	0.757
Alive at end of study period, n (%)	163 (71.81)	44 (81.48)	67 (77.01)	0.283
Age at death, days, median (IQR), mean	133 (36.5– 399.5), 276.30 <sup>b</sup>	182.5 (29– 330), 188.40 <sup>c</sup>	92.5 (52– 181), 130.85 <sup>d</sup>	0.151
ES, exome sequencing				
<sup>a</sup> All p-values from one-way ANOVA; <sup>b</sup> n = 64; <sup>c</sup> n = 10; <sup>d</sup> n = 20				



Table S7. Medical management change, ES cohort

<b>Clinical Impact Category, n (%)</b>	<b>All ES Patients (n = 368)</b>	<b>ES Diagnosed (n = 102)</b>	<b>ES</b>
			<b>Undiagnosed (n = 266)</b>
Subspecialist or Consult Referral	54 (14.67)	35 (34.31)	19 (7.14)
Surveillance Plan	16 (4.35)	12 (11.76)	4 (1.50)
Screening Recommendation	16 (4.35)	6 (5.88)	10 (3.76)
Medication Change	11 (2.99)	8 (7.84)	3 (1.13)
Redirection of Care	6 (1.63)	3 (2.94)	3 (1.13)
Diet Prescription	5 (1.36)	3 (2.94)	2 (0.75)
Total	81 (22.01)	49 (48.04)	32 (12.03)
ES, exome sequencing			

## **Cost-Effectiveness Analysis of Clinical Exome Sequencing Compared to Standard Diagnostics for Critically Ill Infants**

**Target Journal:** *Genetics in Medicine*

### **Abstract**

**Purpose:** Estimate the cost-effectiveness of clinical whole exome sequencing (ES) as a diagnostic tool, compared to usual diagnostic care, for patients with a suspected genetic etiology admitted to intensive care within the first year of life.

**Methods:** In this retrospective cohort study, we analyzed ES application at a children's hospital December 2011 – June 2017. Diagnostic yield and survival were compared between cohorts of clinically similar patients who did (n=368) and did not (n=368) have ES in diagnostic workup, along with total and diagnostic-related costs of index admission and year.

**Results:** Molecular diagnostic yield (25.8% No-ES, 27.7% ES;  $p=0.56$ ) and 1-year survival (84.8% No-ES, 80.2% ES;  $p=0.10$ ) were similar between cohorts, while ES patients had higher cost of admissions, diagnostic investigations, and genetic tests (all  $p<0.01$ ). Incremental diagnostic pathway cost per additional diagnosis was \$550,874 for ES patients, which was reduced to \$46,489 when comparing ES cohort to patients for whom ES was recommended but not performed.

**Conclusion:** ES is an important tool for diagnosing critically ill newborns and infants, as are chromosomal microarray and targeted genetic testing, yet it does not

appear cost-effective during initial clinical uptake. Further work is needed to develop clinical guidelines for appropriate application of ES.

## **Introduction**

Evaluation of patients with suspected genetic disorders is important to pediatric and neonatology practice due to incidence of genetic diseases that manifest at birth or soon after. Neonatal intensive care has been upheld as the setting in which clinical genomic sequencing (cGS) has the most promise for both clinical and economic impact.<sup>1-3</sup> Diagnostic workup for a suspected genetic condition is associated with longer, more costly neonatal intensive care unit (NICU) admissions, and diagnosis of genetic-based disease is associated with longer inpatient stays and higher cost of care compared to other pediatric chronic disease etiologies.<sup>4-6</sup> Based on national estimates, up to 14% of inpatient pediatric admissions are associated with genetic disease, at a cost of up to \$77,000 higher for neonatal admissions and up to \$17,000 for pediatric admissions, compared to patients who did not have a genetic disease diagnosis.<sup>7</sup>

Exome sequencing (ES) has demonstrated ability to identify diagnoses in critically ill newborns and infants, clinical uptake is increasing, and evidence of overall clinical utility is building.<sup>8-12</sup> However, clinical guidelines for ES application are not yet developed. Compared to the current standard genetic diagnostic workup, including chromosomal microarray (CMA) and targeted gene sequencing,<sup>13,14</sup> very little is known about the population-level impact of ES on health outcomes or cost of care. Cost-effectiveness evidence is severely lacking, especially within the US

health care system.<sup>12,15</sup> Economic evaluation can inform appropriate clinical use and patient access to cGS.

Among the few ES economic evaluations to date, the most robust have been based on data generated within a research framework on the efficacy of ES to make molecular diagnoses.<sup>16,17</sup> Although the goal of cost-effectiveness analysis is to assess an intervention's impact in practice rather than a trial environment,<sup>18,19</sup> conclusions of these modeling studies reflect study inclusion criteria, such as clinical presentation restrictions and requirement of previous non-diagnostic tests. Therefore, it may not be possible to extrapolate results to the real-world clinical setting. The impact of clinically applied ES on outcomes and costs compared to usual diagnostics in a large group of patients with suspected genetic disease has not yet been examined.

The aim of this paper is to estimate the cost-effectiveness of ES as a diagnostic tool for infants less than 1 year of life with a suspected genetic etiology in intensive care settings. We study real-world ES application during the initial 5 years of uptake at a large children's hospital by comparing clinically similar cohorts of patients who did and did not have ES as part of a diagnostic workup. We analyze and compare cost of care, diagnostic yield and survival outcomes for both cohorts, and estimate cost-effectiveness using electronic medical record and hospital cost data over the time horizon of the inpatient hospital stay and within one year of the initial Genetics service consultation.

## **Methods**

### ***Study Design***

We analyzed ES application at a large children's hospital located in an academic medical center from December 2011 through June 2017. The study population was undiagnosed patients with a suspected genetic etiology who had an intensive care unit (ICU) admission within the first year of life. Patients were admitted to Texas Children's Hospital (TCH) in Houston, TX, a large children's hospital with 33,000 total admissions in 2017 and the highest level (Level IV) of neonatal intensive care.<sup>20</sup> All TCH ES orders were sent to Baylor Genetics (BG) diagnostic laboratory.

Documentation of genetics consultation in the electronic medical record (EMR) indicated a suspected genetic etiology in the patient. The index admission was defined as the admission during which the initial genetics consult was ordered, and the index year was defined as the 365 days after the initial genetics consult took place. The 1-year time horizon allows estimation of ES impact on care provision and costs, even if results were not returned before index admission discharge.

We employed a retrospective cohort study design in which ES was the exposure factor. Included patients met the following criteria: (1) ICU admission within the first year of life; (2) inpatient genetics consultation documented in the medical record; (3) inpatient stay and genetics consultation occurred between December 1, 2011 and June 30, 2017. The ES cohort consisted of patients who met the following additional criteria: (1) received ES as part of the diagnostic pathway; (2) ES ordered within the first year of life.

Among included patients, patients who did not receive ES were evaluated for inclusion in the comparator cohort (No-ES). Methods for patient matching are detailed elsewhere.<sup>21</sup> Briefly, patients included in the No-ES cohort were those propensity score-matched to ES cohort patients. The propensity score was calculated based on patient's age at first genetics consult (by quarter of year), hospital unit in which the genetic consult occurred (NICU; pediatric intensive care unit, PICU; cardiovascular intensive care unit, CVICU; progressive care unit, PCU; other unit), consult date (by quarter of study period), sex, and phenotypic characteristics (Human Phenotype Ontology terms).

Data on patient characteristics, genetics consultation, and clinical outcomes were collected through retrospective EMR review. ES order and report data were obtained from BG, and cost data were obtained from hospital administrative records. Data analysis was performed using Stata/IC 15.1 (StataCorp, College Station, TX).

### ***Interventions and Comparator***

Patients in the ES cohort had one of three ES forms: sequencing of the patient only (proband ES), sequencing of the patient and both parents (trio ES) and sequencing of the patient and both parents with a reduced turnaround time (critical trio ES). Patients in the No-ES cohort had a diagnostic workup that did not include ES but may have included other forms of genetic testing such as chromosomal microarray (CMA), fluorescence in situ hybridization (FISH), single gene or gene panel sequencing, deletion/duplication analysis, or methylation studies. A clinician may have ordered ES for patients in the No-ES cohort without it being performed for

reasons such as lack of parental consent or cancelation by lab due to insufficient blood sample; we refer to these patients as the ES-recommended group.

### ***Outcomes***

Main outcome measures considered in this study were establishment of a molecular diagnosis and 1-year survival. Molecular diagnosis was defined as the identification of a specific genetic change interpreted by a clinician as the cause or probable cause of the patient's clinical presentation. Subgroup analyses were performed based on patient characteristics intended to generate evidence to inform clinical guideline development and decision-making. Subgroups were: patients admitted in 2016 and 2017 (after all three ES forms were available), patients who survived to 28 days of life, patients in the NICU during the genetics consultation, and patients in the ES-recommended group.

### ***Cost Analysis***

Costs were calculated using the hospital perspective. We analyzed total index admission and index year cost, cost by hospital billing category (UB revenue code), cost of the investigator-defined diagnostic pathway, and cost of genetic tests. To mitigate the influence of length of stay (LOS) on total index admission cost, we calculated the proportion of the total index admission cost accounted for by costs in each billing category, as well as the diagnostic pathway costs and genetic testing costs, at the patient level.

As there is no standard diagnostic pathway for the heterogeneous population of patients in this study, we defined the diagnostic pathway as clinical tests performed for the purpose of making a diagnosis rather than routine care or monitoring. An inclusion rule of “first,” “none,” or “all” was determined for each test on a list of all laboratory and radiology tests performed for study patients. The rule was used to determine which, if any, instance of a specific test on a patient was performed as part of the diagnostic pathway and applied to cost data to sum the cost of diagnostic pathway investigations. Similarly, we identified each genetic test in the list of laboratory tests and applied these rules to the cost data to determine the cost of genetic tests. In each patient’s cost data, each line-item labeled “miscellaneous referred test” was cross-referenced by service date to tests ordered in the EMR as miscellaneous referred tests to determine whether it was a genetic test or not. All tests determined to be genetic tests were included in both the diagnostic pathway and genetic test cost categories. Genetic test and diagnostic pathway costs are not necessarily inclusive or exclusive of the hospital administrative billing categories.

For analysis of cost data, which has a skewed distribution, we used log transformations and non-parametric statistical tests. We used ordinary least squares (OLS) regression on logged total cost of index admission to estimate the impact of patient characteristics on index admission cost. We employed Wilcoxon rank-sum and Kruskal-Wallis tests to compare cost categories between cohorts and ES forms, respectively. Chi-square and Wilcoxon rank-sum tests were used for other comparisons as appropriate. Relevant cost analyses were performed for each subgroup of patients.



We describe cost of index admission, index year, and diagnostic pathway and genetic test during the index admission and the year. We calculated the cost of the index admission and the diagnostic pathway per diagnosis and percent 1-year survival. We calculated incremental cost-effectiveness ratios (ICERs) for incremental index admission diagnostic pathways costs and incremental diagnoses, and for incremental index admission genetic test costs and incremental diagnoses. For each ICER, 95% confidence intervals were constructed from 1,000 bootstrap replicates.<sup>22</sup> All costs were adjusted to 2017 USD\$ using the historical Consumer Price Index for All Urban Consumers (CPI-U): U.S. city average per year.

## **Results**

### ***Outcomes***

The patient matching process successfully reduced variation in observable characteristics between patients in the No-ES and ES cohorts. Outcomes are reported elsewhere in more detail.<sup>21</sup> Characteristics of the patient population are given in Table 1. Molecular diagnostic yield (25.8% No-ES, 27.7% ES;  $p=0.56$ ) and 1-year survival (84.8% No-ES, 80.2% ES;  $p=0.10$ ) were similar for both cohorts (Table 2). In the ES cohort, 8 additional diagnoses were made with CMA not included in the ES diagnostic yield. Among patients admitted in 2016 and 2017, molecular diagnostic yield ( $p=0.02$ ) was higher in the ES cohort than the No-ES cohort, as there was lower yield in the No-ES cohort over this period (Table 3).

In the ES cohort, ES resulted before discharge from the index admission for 106 (28.8%) patients. A significantly higher proportion of patients who had critical trio

were diagnosed before discharge (24.1%) than patients who had proband or trio tests (5.7%,  $p<0.01$ ). Prior to the lab reporting results, 46 (12.5%) patients were deceased, and 11 patients who expired while in the hospital later had a diagnostic finding. For ES results communicated after patient discharge, results were returned 121 days (median) after discharge.

### **Costs**

Distributions of total cost of index admission and index year by cohort are presented in Figure 1 and Figure 2, respectively. Index admission total cost, diagnostic pathway cost, and genetic test cost were higher for ES patients than No-ES patients (Table 2). Cost of the index admission diagnostic pathway per diagnosis were \$24,763 [95% bootstrapped CI \$19,956—\$29,569] for the No-ES cohort and \$60,869 [\$49,749—\$71,953] for the ES cohort. ES cohort Incremental index admission diagnostic pathway cost per additional diagnosis was \$550,874. The incremental cost of index admission genetic tests per additional diagnosis was \$410,614.

Index year total cost, diagnostic pathway cost, and genetic test cost were also higher for the ES cohort. Controlling for length of stay and other features of hospitalization, ES was associated with approximately 17.7% higher total cost of index admission ( $p<0.01$ , Table S1). Within the ES cohort, genetic diagnosis was associated with higher index admission costs ( $p<0.01$ ).

Subgroup analyses showed similar results. Among patients admitted in 2016 and 2017, costs in all categories (total, diagnostic pathway, and genetic tests) were

significantly higher in the ES cohort (Table 3). For patients who survived longer than 28 days, costs were higher in the ES cohort in all categories (Table S2). Among patients with genetics consultation in the NICU, costs were significantly higher in the ES cohort (Table S3). Within the ES cohort, total cost of the index admission and index year were not significantly different between patients with each form of ES, but the cost of diagnostic pathway and genetic testing was significantly higher for patients with critical trio ES (Table S4).

Within the No-ES cohort, there were 36 patients for whom ES was recommended but not performed. Table 4 compares costs for the ES-recommended group and ES cohort. Molecular diagnostic yield was significantly higher in the ES cohort (102, 27.7%) than the ES-recommended but not performed group (2, 5.6%). This difference in effectiveness results in a lower incremental cost per diagnosis. Therefore, even though costs were higher in the ES group, the incremental diagnostic pathway cost per additional diagnosis was lower for ES-recommended than for other comparator groups at \$46,489. The incremental genetic test cost per additional diagnosis was \$36,246.

A comparison of cost components of the index admission is presented in Table 5 and Figure 3. As a proportion of the index admission total cost, cost of the diagnostic pathway was higher for the ES cohort (12.7%) than the No-ES cohort (7.9%,  $p<0.001$ ). Similarly, genetic tests accounted for 8.4% of the index stay cost in the ES cohort and 3.7% in the No-ES cohort ( $p<0.001$ ). By billing category, diagnostic UB revenue code cost was not different between cohorts, but laboratory cost made up a larger share of admission cost in the ES cohort (15.2%) than the No-

ES cohort (9.9%,  $p < 0.001$ ). Nursing care accounted for the largest share of index admission total cost in both cohorts, although it represented a smaller share in the ES cohort (56.2%) than the No-ES cohort (60.7%,  $p < 0.001$ ). Pharmacy, radiology, and therapeutic billing categories made up similar shares of admission cost in both cohorts (all  $p > 0.58$ ).

Diagnoses of 4 conditions were made in both cohorts: CHARGE syndrome, Noonan syndrome, Walker-Warburg syndrome, and Gaucher disease. Mean cost of the diagnostic pathway and genetic tests, as well as the total cost of the index admission and year, were higher for patients with the same ultimate diagnosis in the ES cohort for each diagnosis (Table S5; individual data not shown). Average length of index admission stay was longer for patients in the ES cohort than patients with the same diagnosis in the No-ES cohort for 3 of the 4 diagnoses.

## **Discussion**

Our findings suggest that ES is not cost-effective, in terms of summary effect measures of diagnostic yield and percent survival, as applied during the initial 5 years of clinical uptake at one large academic children's hospital. ES patients had a longer and more costly index inpatient admission than clinically similar patients who did not have ES, on average. Our broad patient population of newborns and infants with suspected genetic disease allowed analysis of the real-world impact of ES on costs. Our findings suggest that ES is not cost-effective when applied generally to critically ill patients with suspected genetic disease presentations within the first year of life. Because the contribution of large chromosome structural rearrangements and

gene copy number variations present in this population, chromosomal analyses identified a diagnosis in a roughly equal proportion of patients and were associated with lower costs.

Although the number of diagnoses out of the total number of patients in each cohort was similar, the diagnoses made in each cohort were qualitatively different based on the types of changes identifiable by tests used in each group (e.g., structural changes in the No-ES cohort versus single-gene sequence variants in the ES cohort). Some patients in the No-ES cohort may not have had ES because the clinician determined ES was not necessary or was the inappropriate genetic diagnostic tool to use.

However, for some patients in the No-ES cohort, not having ES was the result of factors external to the clinical decision-making process, which makes these patients plausibly the best comparison group to ES patients. In these cases where ES was recommended but not performed, diagnostic yield was significantly lower than in the ES cohort. Among all of our subgroup analyses, the ICER for diagnosis is most favorable when comparing the ES cohort to the ES-recommended group of No-ES cohort patients. Comparing diagnostic pathway cost in these two groups, an incremental cost of \$46,489 per additional diagnosis may be deemed reasonable by decision makers, especially considering the backdrop of the high cost of admission for these patients.

Our identifying assumption that a similar patient may have had ES or not depending upon who performed the consultation was supported by findings in prior work in which we find evidence of variability in ES uptake among attending

geneticists.<sup>21</sup> However, we cannot rule out the potential, and results reported herein suggest, that ES was applied with bias toward more complex patients overall. In the absence of formal guidelines for ES use, it is difficult to map out details of the clinical decision-making process regarding ES order, yet it appears that clinicians have recommended ES in appropriate patients, all things considered. A formal characterization of this population from a phenotype and clinical presentation perspective, using more nuanced details than we were able to capture retrospectively through HPO terms, should be the subject of future work to develop guidelines for use in other institutions that may have less prior research-based familiarity with ES than academic physicians ordering ES in this study.

Research that has found ES cost-effective has identified patient populations in which ES is the correct test by, for example, requiring clinician consensus, study enrollment, prerequisite non-diagnostic tests, and by using parallel, prospective study design to analyze the diagnostic pathway with and without cGS for the same patients.<sup>16,23</sup> Results from a cost-effectiveness modeling study in an Australian infant cohort suggest that ES performed as a first-line test may be cost-saving.<sup>16</sup> Evaluating costs in such a setting is analogous to economic evaluation conducted alongside a randomized controlled trial in that there are threats to external validity because ES is applied as appropriately as possible. From a payer perspective, Sabatini and colleagues demonstrated that whether use of ES was cost-saving or cost-increasing depended upon clinical features of the patient population, the cost of the test and where it was incorporated in the diagnostic pathway.<sup>24</sup> Other studies have summed costs of non-diagnostic workup prior to diagnostic ES,<sup>25-27</sup> that can be

informative descriptions but lack a necessary comparator group for formal cost-effectiveness analysis.

We make a different contribution than studies of controlled ES application by evaluating impact for a broadly defined patient population. Our approach was optimal in order to utilize information on the large number of patients who had already had ES, ensure follow-up time of at least one year over which to measure outcomes, and enable comparison of multiple outcomes. The time frame of the study shows early adoption and implementation in the absence of clinical guidelines about application. Although uptake was variable during early stages of implementation, it is because of this property that we are able to construct a clinically similar comparison cohort that did not have ES before ES becomes standard-of-care. In the already high-cost setting of the NICU, availability of technology should not replace careful clinical assessment as to whether the patient is a good candidate for ES or not. CMA and other chromosomal tests remain important diagnostic tools for this patient population given the substantial burden of chromosomal abnormalities for children under one year of age in the United States.<sup>28,29</sup>

Results from the ES-recommended group can support the hypothesis that ES might be most cost-effective when applied in patients that are difficult or impossible to diagnose with other modalities. This speaks to the original intended use of ES upon its availability as a clinical test for patients characterized as “hard to diagnose” and for whom other tests such as CMA are non-diagnostic. Clearer definition of how to identify such patients and quicker sequencing turnaround is needed to save cost.

## ***Limitations***

Our study is limited by reliance on outcome data and clinical notation documented in the EMR, which may not be comprehensive. Administrative cost data, although reflective of the billing process, may not be entirely accurate or consistent. For example, there was variability in clinicians' ES order entry in the EMR and how ES costs appeared in the cost data. This variability is a hurdle to performing a sensitivity analysis of the cost of care without ES in the ES cohort, and the degree to which higher costs for the ES cohort are attributable only to the cost of ES will be explored further. We are not able to account for care patients received at other institutions unrecorded in our EMR and administrative system. While this does not impact our ability to analyze the index admission, we may not capture all relevant costs over the index year.

Results of this study cannot be used to draw conclusions about optimal placement of ES within the diagnostic pathway, such as use as a first-line test or subsequent other non-diagnostic investigations. This analysis only examines pathways with and without ES; it does not attempt to quantify cost-effectiveness for ES at a particular point in the diagnostic pathway. Lack of established practice guidelines on ES makes choice of comparators difficult because each provider may order sequencing based on different criteria or at a different point in the diagnostic pathway, and cost-effectiveness is always dependent upon the clinical context.

The majority of ES patients did not have ES results returned during the index admission, meaning there was little potential for impact on care provision or the associated cost that would be captured in the cost of the index admission. Some of



the largest estimated cost savings as a result of neonatal cGS have come from saving inpatient days in hypothetical counterfactual cases when rapid sequencing results are returned while patients are admitted (median 23 days from order).<sup>30</sup> Because not all ES are ordered as a rapid test, we study the one-year follow-up period to allow return of results and potential for impact on care. For lengthy index admissions, the index admission almost entirely eclipses the index year. However, for patients with shorter, more frequent admissions and clinic visits, we are able to capture more of the ES impact. The higher costs for the index year ES group mirror the higher index admission costs. If return of ES results impacted care over the longer term, we would expect the cost of the index year to be proportionately higher than the index admission for the No-ES group.

To address the heterogeneity of the patient population, patients were matched on phenotypic and clinical characteristics, which successfully reduced observable differences between the cohorts. Even so, the underlying cause of disease remained diverse within cohorts. Unless each molecular diagnosis is modeled separately, which is not practical for sample size reasons, patient groupings will necessarily combine individuals with different genetic etiology. Heterogeneity presents challenges for modeling costs over a longer time horizon than the inpatient stay because each distinct molecular diagnosis will have different prognostic and treatment trajectories. One interpretation of the insignificant difference in survival supports that the patients were well-matched in severity. However, more nuanced observations of diagnoses, costs, and clinical course (using

LOS as a proxy) suggests ES patients may still differ in some unexplained way from No-ES patients, on average, even for patients with the same ultimate diagnosis.

While diagnostic yield was similar for ES and No-ES patients, we are not able to address differences in importance of availability of ES as a diagnostic tool with the ability to test for and detect rare disorders that it, among clinical tests, can uniquely identify. As highlighted by 2 recent systematic literature reviews of genomic sequencing, there is a need for more systematic outcomes measurement to enable health services research and economic evaluation of genomic sequencing.<sup>12,15</sup> An important consideration is the potential utility of ES results – both diagnostic and non-diagnostic – and implications for the patient and patient’s family that may fall outside the realm of medical actionability. Number of diagnoses made does not necessarily convey the perceived utility of the results for either clinical decision-making or the patient’s family. Clinicians may use ES results differently than results from other diagnostic tests, an unremarkable ES result that does not pinpoint a diagnosis may be more important than non-diagnostic results from other investigations, and clinicians may perceive outcomes outside of the traditional notion of medical management changes to be important results of ES.<sup>31</sup> Moreover, families value genetic information for personal as well as medical purposes.<sup>32</sup> Further development of methods to measure clinical utility, in a broader sense, of cGS is warranted.

There are possible 5 channels to increase cost-effectiveness of ES. First, reduced turnaround time for results, which would allow greater potential for impact on LOS and medical management. Second, development of clinical guidelines to

rule out certain kinds of diagnoses first, such as a requirement of non-diagnostic CMA, prior to ES order. Third, reanalysis of genetic data and a longer time horizon over which to measure costs, which can lead to increased diagnostic yield upon reanalysis of the patient's previously generated sequence information and potential impact on care.<sup>33,34</sup> Fourth, reduction of ES cost, which may be possible with increased automation of result interpretation. Fifth, consideration of broader effects of ES which may flow from both diagnostic and non-diagnostic results. Systematic categorization and documentation of changes in medical management as a result of ES results to provide a robust description of utility would advance work in this area. Development of tools to measure utility to the patient and family should also be investigated.

## ***Conclusion***

ES demonstrated important diagnostic utility for patients with monogenic disease, yet other genetic tests, especially chromosomal microarray, remain important given the burden of chromosomal abnormalities in this population. As clinically applied over the first 5 years, ES does not appear cost-effective as a diagnostic tool for the broad population of newborns and infants with suspected genetic disease. Further work is needed to develop outcome measures to capture utility of both diagnostic ES results and non-diagnostic ES results for clinicians, patients, and patients' families and to specify clinical guidelines for appropriate ES application.

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## Tables and Figures

Table 1. Patient Characteristics

	No-ES Cohort	ES Cohort	p-value <sup>a</sup>
Sex, n (%)			
Male	203 (55.16)	217 (58.97)	
Female	165 (44.84)	151 (41.03)	0.297
Race, n (%)			
White/Caucasian	277 (75.27)	289 (78.53)	
Black/African-American	58 (15.76)	58 (15.76)	
Asian	22 (5.98)	17 (4.62)	
American Indian and Alaska Native	1 (0.27)	1 (0.27)	
Unknown	8 (2.17)	3 (0.82)	0.396
Ethnicity, n (%)			
Non-Hispanic	200 (54.35)	190 (51.63)	
Hispanic	162 (44.02)	174 (47.28)	
Unknown	6 (1.63)	4 (1.09)	0.581
Preferred Language, n (%)			
English	312 (84.78)	294 (79.89)	
Spanish	52 (14.13)	69 (18.75)	
Other	4 (1.09)	5 (1.36)	0.219
Unit of Genetics Consult, n (%)			

NICU	245 (66.58)	222 (60.33)	0.078
CVICU	62 (16.85)	70 (19.02)	0.442
Other	36 (9.78)	36 (9.78)	1.00
PCU	17 (4.62)	25 (6.79)	0.204
PICU	8 (2.17)	15 (4.08)	0.138
Age at Genetics Consult (quartile of year), n (%)			
First	302 (82.07)	297 (80.71)	0.636
Second	43 (11.68)	49 (13.32)	0.504
Third	13 (3.53)	12 (3.26)	0.839
Fourth	10 (2.72)	10 (2.72)	1.00
Genetics Consult Date (quartile of study period), n (%)			
First	92 (25.00)	92 (25.00)	1.00
Second	105 (28.53)	79 (21.47)	0.027
Third	90 (24.46)	95 (25.82)	0.671
Fourth	81 (22.01)	102 (27.72)	0.073
Gestational Age at Birth, weeks, mean (median)	36.40 (37.29)	37.00 (38.00)	0.0711 <sup>b</sup>
Mother's Age at Birth, years, mean (sd)	28.93 (6.27) <sup>d</sup>	28.39 (6.33) <sup>e</sup>	0.251 <sup>c</sup>
Father's Age at Birth, years, mean (sd)	31.60 (7.55) <sup>f</sup>	31 (7.78) <sup>g</sup>	0.357 <sup>c</sup>
ES, exome sequencing; NICU, neonatal intensive care unit; CVICU, cardiovascular intensive care			

unit; PCU, progressive care unit; PICU, pediatric intensive care unit

<sup>a</sup> All p-values from chi-square tests unless otherwise noted; <sup>b</sup> Wilcoxon rank-sum test <sup>c</sup> Student's t-test; <sup>c</sup> n = 360; <sup>d</sup> n = 362; <sup>e</sup> n = 312; <sup>f</sup> n = 316

Table 2. Costs by cohort<sup>a</sup>

	<b>All Patients (n = 736)</b>	<b>No-ES Cohort (n = 368)</b>	<b>ES Cohort (n = 368)</b>	<b>p-value</b>
Molecular diagnosis, n (%)	205 (27.85) <sup>b</sup>	95 (25.82)	102 (27.72)	0.560 <sup>c</sup>
Survival to 28 days, n (%)	686 (93.21)	337 (91.58)	349 (94.84)	0.079
Survival to 1 year, n (%)	607 (82.47)	312 (84.78)	295 (80.16)	0.099
Total cost of index admission, mean (sd)	272,600 (401,499)	218,503 (338,489)	326,698 (449,887)	< 0.001 <sup>d</sup>
Total cost of index year, mean (sd)	338,179 (421,266)	266,768 (357,009)	409,591 (466,617)	< 0.001 <sup>d</sup>
Total cost of index admission diagnostic pathway, mean (sd)	11,632 (9,429)	6,393 (5,243)	16,871 (9,773)	< 0.001 <sup>d</sup>
Total cost of index year diagnostic pathway, mean (sd)	13,886 (10,398)	7,584 (6,018)	20,188 (10,034)	< 0.001 <sup>d</sup>

Total cost of index genetic tests, mean (sd)	6,322 (6,533)	2,417 (2,905)	10,227 (6,817)	< 0.001 <sup>d</sup>
Total cost of index year genetic tests, mean (sd)	7,239 (7,265)	2,627 (3,633)	11,851 (7,061)	< 0.001 <sup>d</sup>
Cost (index admission) per percent 1-year survival [95% CI] <sup>e</sup>	948,413 [779,806—1117022]	1,499,755 [1,251,970—1,747,540]		
Cost (index year) per percent 1-year survival [95% CI] <sup>e</sup>	1,157,910 [9,77,368—1,338,451]	1,880,285 [1,606,999—2,153,570]		
Cost (index admission diagnostic pathway) per diagnosis, [95% CI] <sup>e</sup>	24,763 [19,956—29,569]	60,869 [49,784—71,953]		
Incremental cost-effectiveness ratio for index admission diagnostic pathway diagnoses [95% CI] <sup>e</sup>	550,874 [-5,651,018—6,752,766]			
Incremental cost-effectiveness ratio for index admission genetic testing diagnoses [95% CI] <sup>e</sup>	410,614 [-4,106,198—4,927,426]			
ES, exome sequencing <sup>a</sup> All costs reported in 2017 USD\$ <sup>b</sup> Additional 8 diagnoses made by chromosomal microarray in ES cohort not included in ES diagnostic yield <sup>c</sup> chi-square test; <sup>d</sup> Wilcoxon rank-sum test <sup>e</sup> 95% CI, confidence intervals constructed from 1,000 bootstrap replicates				

Table 3. Costs, Patients admitted in 2016 and 2017 <sup>a</sup>				
	<b>All Patients (n = 257)</b>	<b>No-ES Cohort (n = 119)</b>	<b>ES Cohort (n = 138)</b>	<b>p-value</b>
Molecular diagnosis, n (%)	61 (23.74) <sup>b</sup>	19 (15.97)	39 (28.26)	0.019 <sup>c</sup>
Survival to 28 days, n (%)	233 (90.66)	102 (85.71)	131 (94.93)	0.011 <sup>c</sup>
Survival to 1 year, n (%)	206 (80.16)	92 (77.31)	114 (82.61)	0.288 <sup>c</sup>
Total cost of index admission, mean (sd)	280,491 (370,566)	217,571 (273,630)	334,747 (430,933)	0.003 <sup>d</sup>
Total cost of index year (mean)	338,516 (389,571)	256,202 (294,373)	409,497 (444,964)	<0.001 <sup>d</sup>
Total cost of index admission diagnostic pathway, mean (sd)	13,048 (8,389)	6,358 (4,249)	18,818 (6,599)	<0.001 <sup>d</sup>
Total cost of index year diagnostic pathway, mean (sd)	14,585 (8,912)	7,483 (5,107)	20,708 (6,661)	<0.001 <sup>d</sup>
Total cost of index genetic tests, mean (sd)	7,671 (5,850)	2,395 (2,206)	12,221 (3,838)	<0.001 <sup>d</sup>

Total cost of index year genetic tests, mean (sd)	8,113 (5,921)	2,749 (2,831)	12,739 (3,472)	<0.001 <sup>d</sup>
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ES, exome sequencing

<sup>a</sup> All costs reported in 2017 USD\$

<sup>b</sup> Additional 2 diagnoses by chromosomal microarray in ES cohort not included in ES diagnostic yield

<sup>c</sup> chi-square test

<sup>d</sup> Wilcoxon rank-sum test

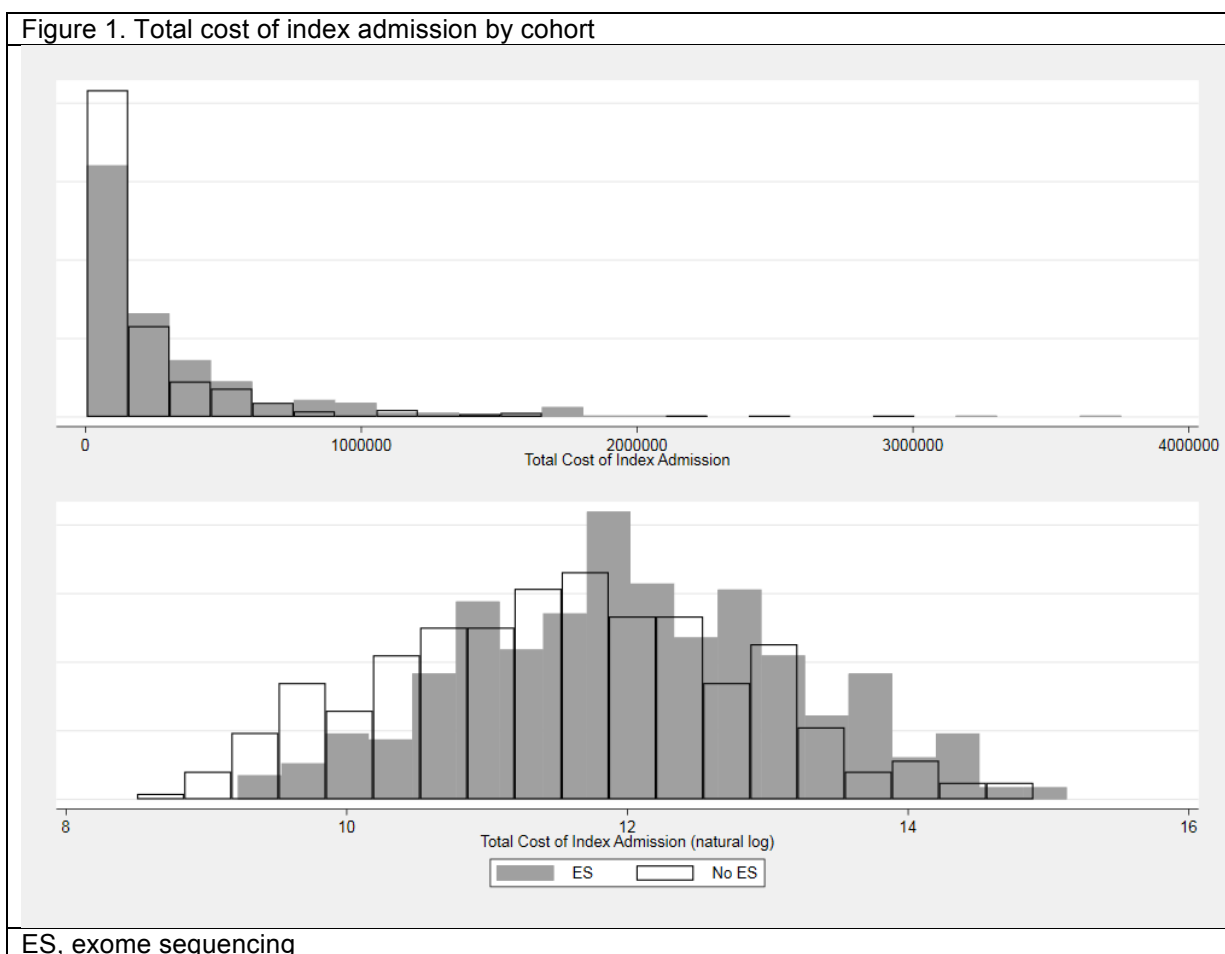
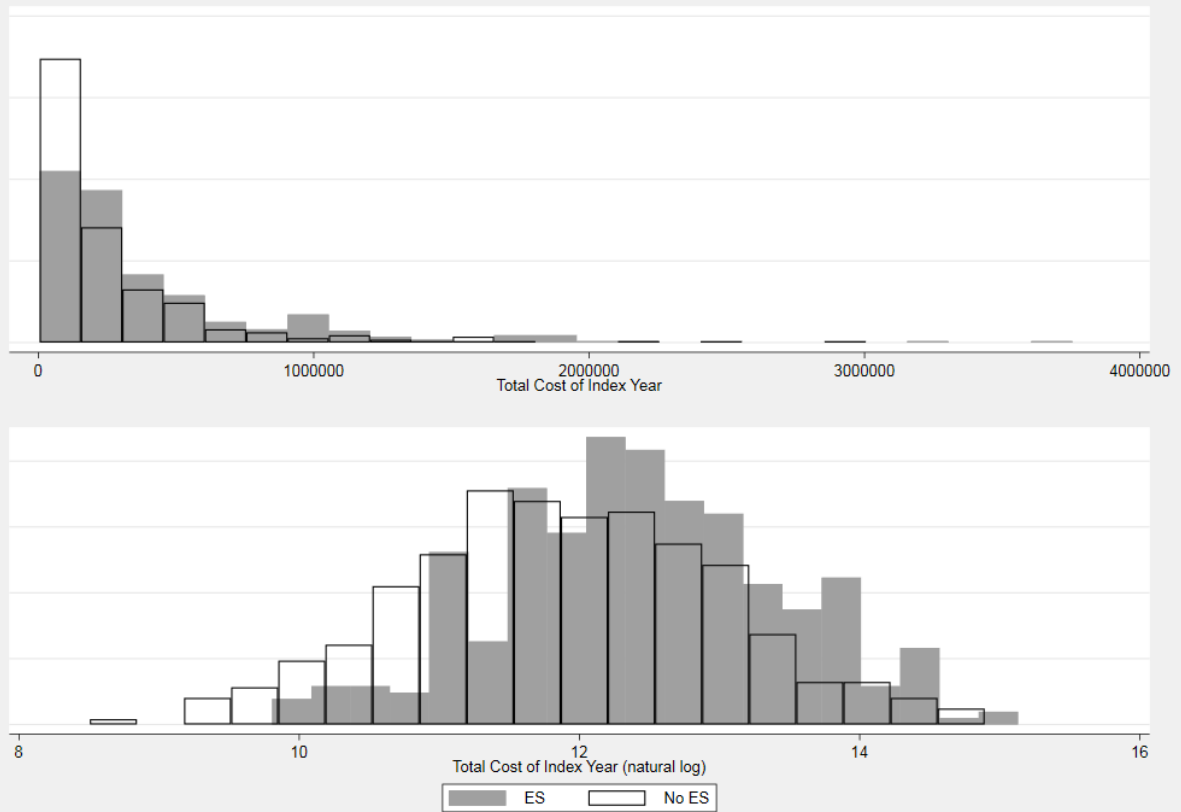


Figure 2. Total cost of index year by cohort



ES, exome sequencing

Table 4. Costs by ES recommended but not performed <sup>a</sup>			
	<b>No-ES Cohort, ES recommended (n = 36)</b>	<b>ES Cohort (n = 368)</b>	<b>p-value<sup>b</sup></b>
Molecular diagnosis, n (%)	2 (5.56)	102 (27.72)	0.004 <sup>c</sup>
Survival to 28 days, n (%)	32 (88.89)	349 (94.84)	0.142
Survival to 1 year, n (%)	28 (77.78)	295 (80.16)	0.733
Length of stay, median (IQR), mean	35.5, (9.5—58), 43.28	39 (17—83.5), 66.87	0.107
Total cost of index admission, mean (sd)	178,135 (172,766)	326,698 (449,887)	0.057
Total cost of index year, mean (sd)	246,097 (263,362)	409,591 (466,617)	0.008
Total cost of index admission diagnostic pathway, mean (sd)	6,568 (5,087)	16,871 (9,773)	<0.001
Total cost of index year diagnostic pathway, mean (sd)	7,704 (5,147)	20,188 (10,034)	<0.001
Total cost of index genetic tests, mean (sd)	2,194 (3,000)	10,227 (6,817)	<0.001
Total cost of index year genetic tests, mean (sd)	2,450 (3,373)	11,851 (7,061)	<0.001
Incremental cost-effectiveness ratio for index admission diagnostic pathway diagnoses [95% CI] <sup>d</sup>		46,489 [16,701—76,278]	
Incremental cost-effectiveness ratio for index admission genetic testing diagnoses [95% CI] <sup>d</sup>		36,246 [14,578—57,915]	



ES, exome sequencing; IQR, interquartile range

<sup>a</sup> All costs reported in 2017 USD\$

<sup>b</sup> Wilcoxon rank-sum test unless otherwise noted; <sup>c</sup> chi-square test

<sup>d</sup> 95% CI, confidence intervals constructed from 1,000 bootstrap replicates

Table 5. Cost drivers, index admission<sup>a</sup>

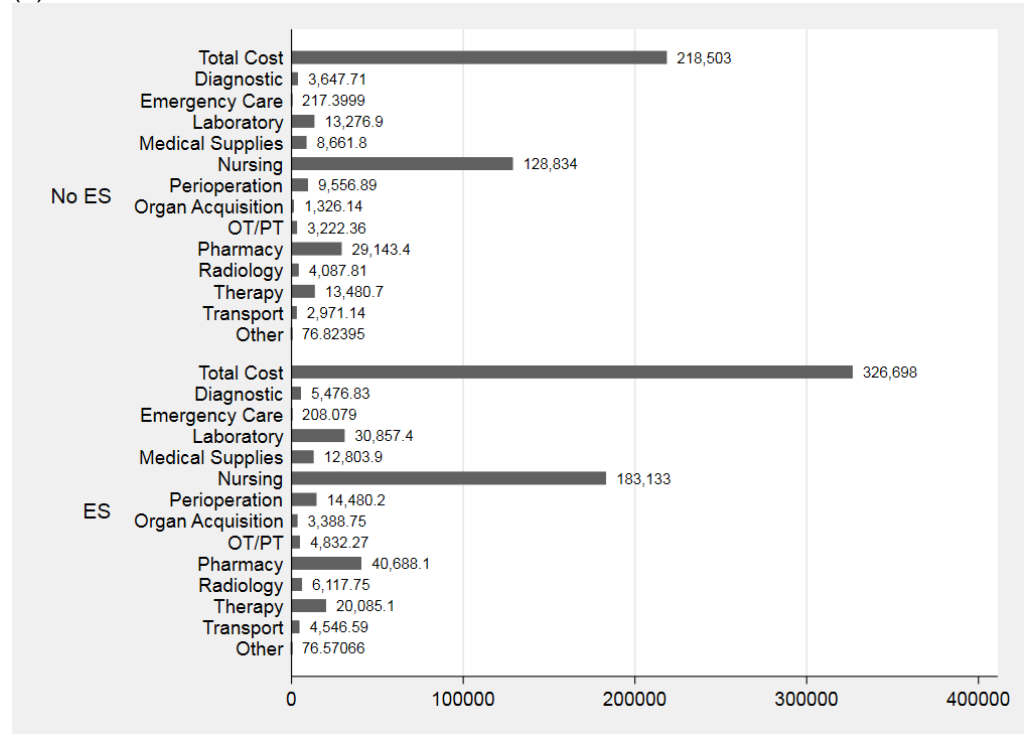
<b>Cost Category<sup>b</sup></b>	<b>Any cost, n</b>	<b>All patients with any cost, mean (sd)</b>	<b>All patients, mean (sd) (n = 736)</b>	<b>Mean % admission cost, all Patients</b>	<b>No-ES Cohort, mean (sd) (n = 368)</b>	<b>Mean % admission cost, No ES Cohort</b>	<b>ES Cohort, mean (sd) (n = 368)</b>	<b>Mean % admission cost, ES Cohort</b>	<b>p- value<sup>c</sup></b>
Diagnostic Pathway	736	11,632 (9,429)	11,632 (9,429)	10.33	7,584 (6,018)	7.94	20,188 (10,034)	12.73	< 0.001
Genetic Tests	736	6322 (6,534)	6,322 (6,534)	6.08	2,417 (2,905)	3.73	10,227 (6,817)	8.43	< 0.001
Diagnostic	714	4,703 (6,475)	4,562 (6,428)	2.42	3,647 (5,691)	2.44	5,477 (6,976)	2.41	0.715
Emergency Care	138	1,135 (365)	213 (4,70)	0.47	217 (481)	0.59	208 (460)	0.35	0.796
Laboratory	736	22,067 (32,051)	22,067 (32,051)	12.55	13,277 (20,014)	9.88	30,857 (38,754)	15.21	< 0.001
Medical/Surgical Supplies	709	11,142 (22,816)	10,733 (22,491)	3.22	8,662 (16,834)	3.27	12,803 (26,853)	3.16	0.285
Nursing Care	736	155,983 (222,073)	155,983 (222,073)	58.43	128,833 (193,753)	60.65	183,133 (244,435)	56.20	< 0.001

Operating Room									
Perioperation Services	616	14,360 (23,061)	12,019 (21,752)	4.52	9,557 (15,620)	4.60	14,480 (26,296)	4.44	0.038
Organ Acquisition	13	133,468 (20,665)	2,357 (17,790)	0.31	1,326 (12,848)	0.21	3,389 (21,601)	0.41	0.163
Occupational/Physical Therapy	595	4,982 (6,419)	4,027 (6,095)	1.64	3,222 (4,938)	1.75	4,832 (6,980)	1.63	0.313
Pharmacy	736	34,916 (94,333)	34,916 (94,333)	7.93	29,143 (93,880)	8.05	40,688 (94,560)	7.82	0.600
Radiology	725	5,180 (6,094)	5,103 (6,081)	2.75	4,088 (5,012)	2.72	6,118 (6,847)	2.77	0.582
Therapeutic	659	18,744 (44,974)	16,783 (42,939)	4.03	13,481 (33,989)	4.07	20,085 (50,154)	4.00	0.861
Transport	3	18,816 (44,974)	77 (1,373)	0.06	77 (1,272)	0.05	77 (1,469)	0.07	0.565
Other	700	3,952 (5,995)	3,758 (5,909)	1.66	2,971 (4,259)	1.82	4,547 (7,109)	1.50	0.553

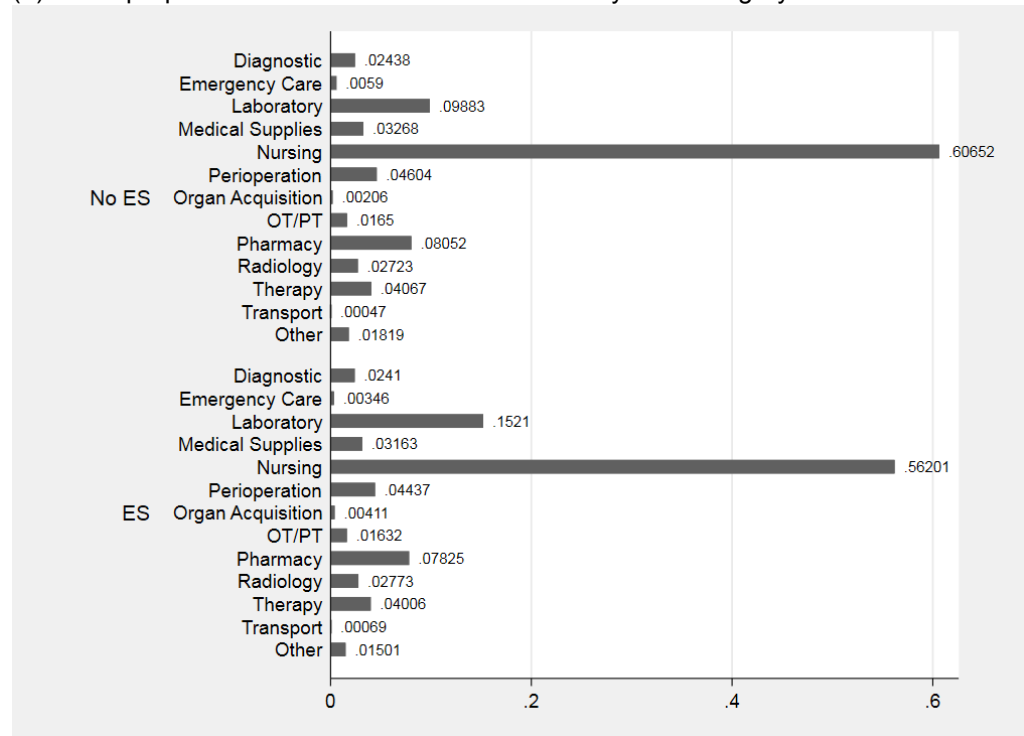
ES, exome sequencing; <sup>a</sup> All costs reported in 2017 USD\$; <sup>b</sup> Cost categories other than Diagnostic Pathway and Genetic Test are by UB Revenue Code; <sup>c</sup> Wilcoxon rank-sum test

Figure 3. Mean index admission total cost by cost category and cohort

(a) Dollar amount



(b) Mean proportion of total index admission cost by cost category and cohort



ES, exome sequencing

**Cost-Effectiveness Analysis of Clinical Whole Exome Sequencing Compared  
to Standard Diagnostics for Critically Ill Infants**

**Supplementary Appendices**

Table S1. Cost of Index Admission Regression Model			
	(1)	(2)	(3)
ln(total cost index admission)	All Patients	No-ES Cohort	ES Cohort
Exome Sequencing	0.163***		
	(0.045)		
Length of Stay	0.020***	0.022***	0.022***
	(0.001)	(0.001)	(0.001)
Length of Stay <sup>2</sup>	-0.00002***	-0.00002***	-0.00003***
	(0.000)	(0.000)	(0.000)
Molecular Diagnosis	-0.122**	-0.094	-0.182***
	(0.051)	(0.079)	(0.059)
DOL Genetics consult	0.0006	0.001	0.001
	(0.000)	(0.001)	(0.000)
Unit <sup>a</sup>			
CVICU	0.545***	0.599***	0.454***
	(0.062)	(0.096)	(0.071)
PICU	0.748***	0.470	0.860***
	(0.145)	(0.294)	(0.141)
PCU	0.230**	0.371**	0.240**
	(0.108)	(0.183)	(0.117)
Other Unit	0.055	0.035	-0.013
	(0.092)	(0.143)	(0.108)
Point of Origin <sup>b</sup>			
Transfer Center	0.026	0.023	0.022

	(0.052)	(0.080)	(0.060)
Self Referral	-0.088	-0.111	-0.092
	(0.086)	(0.130)	(0.102)
Clinic or Physician Referral	-0.407***	-0.628***	-0.203*
	(0.096)	(0.153)	(0.109)
Constant	10.647***	10.555***	10.806***
	(0.051)	(0.073)	(0.057)
n	736	368	368
R-squared	0.767	0.734	0.829
RMSE	0.598	0.651	0.489
<sup>a</sup> NICU base category; <sup>b</sup> Inborn base category Standard errors in parentheses *** p<0.01, ** p<0.05, * p<0.1			

Table S2. Costs by 28-day survival						
	<b>Survived to 28 days (n = 686)</b>	<b>Did not survive to 28 days (n = 50)</b>	<b>p-value<sup>a</sup></b>	<b>No-ES Cohort, Survived to 28 days (n = 337)</b>	<b>ES Cohort, Survived to 28 days (n = 349)</b>	<b>p-value</b>
Molecular diagnosis, n (%)	187 (27.26) <sup>b</sup>	16 (32.00)	0.387 <sup>c</sup>	85 (25.22)	96 (27.51)	0.497
Length of stay, median (IQR), mean	36 (15–71), 62.44	6 (3–10), 7.56	<0.001	29 (12— 59), 54.49	43 (19— 83), 70.13	<0.001
Total cost of index admission, mean (sd)	285,433 (410,426)	84,673 (71,592)	<0.001	229,052 (346,186)	339,874 (458,052)	<0.001
Total cost of index year, mean (sd)	355,510 (428,858)	100,393 (17,0197)	<0.001	281,397 (364,304)	427,076 (472,681)	<0.001
Total cost of index admission diagnostic pathway,	11,871 (9,602)	8,351 (5,730)	0.026	6,508 (5,367)	17,050 (9,946)	<0.001



mean (sd)						
Total cost of index year diagnostic pathway, mean (sd)	14,289 (10,548)	8,351 (5,730)	<0.001	7,808 (6,156)	20,547 (10,123)	<0.001
Total cost of index genetic tests, mean (sd)	6,397 (6,639)	5,291 (4,807)	0.580	2,416 (2,935)	10,240 (6,948)	<0.001
Total cost of index year genetic tests, mean (sd)	7,381 (7,395)	5,291 (4,807)	0.132	2,647 (3,716)	11,952 (7,187)	<0.001
<p>ES, exome sequencing; IQR, interquartile range</p> <p><sup>a</sup> Wilcoxon rank-sum test unless otherwise noted;</p> <p><sup>b</sup> Additional 6 diagnoses made by chromosomal microarray in ES cohort not included in ES diagnostic yield</p> <p><sup>c</sup> chi-square test</p>						

Table S3. Costs, NICU patients only <sup>a</sup>				
	<b>All Patients (n = 467)</b>	<b>No-ES Cohort (n = 245)</b>	<b>ES Cohort (n = 180)</b>	<b>p-value</b>
Molecular diagnosis, n (%)	120 (25.70) <sup>b</sup>	59 (24.08)	56 (25.23)	0.775 <sup>c</sup>
Survival to 28 days, n (%)	430 (92.08)	221 (90.20)	209 (94.14)	0.115 <sup>c</sup>
Survival to 1 year, n (%)	387 (82.87)	207 (84.49)	180 (81.08)	0.329
Total cost of index admission, mean (sd)	214,503 (290,247)	176,466 (254,405)	256,480 (320,630)	<0.001 <sup>d</sup>
Total cost of index year (mean)	268,196 (312,489)	223,439 (285,002)	317,590 (333,997)	<0.001 <sup>d</sup>
Total cost of index admission diagnostic pathway, mean (sd)	10,435 (9,133)	5,463 (3,919)	15,922 (10,064)	<0.001 <sup>d</sup>
Total cost of index year diagnostic pathway, mean (sd)	12,480 (10,047)	6,451 (4,480)	19,135 (10,292)	<0.001 <sup>d</sup>
Total cost of index genetic tests, mean (sd)	6,134 (6,607)	2,387 (2,800)	10,269 (7,117)	<0.001 <sup>d</sup>

Total cost of index year genetic tests, mean (sd)	6,990 (7,219)	2,469 (2,930)	11,981 (7,262)	<0.001 <sup>d</sup>
<sup>a</sup> All costs reported in 2017 USD\$ <sup>b</sup> Additional 5 diagnoses made by chromosomal microarray in ES cohort not included in ES diagnostic yield <sup>c</sup> chi-square test; <sup>d</sup> Wilcoxon rank-sum test				

Table S4. Costs by form of ES <sup>a</sup>				
	<b>Proband ES (n = 227)</b>	<b>Trio ES (n = 54)</b>	<b>Critical Trio ES (n = 87)</b>	<b>p-value</b>
Molecular diagnosis, n (%)	61 (26.87)	13 (24.07)	28 (32.18)	0.523 <sup>b</sup>
Survival to 28 days, n (%)	213 (93.83)	52 (96.30)	84 (96.55)	0.079
Survival to 1 year, n (%)	182 (80.18)	45 (83.33)	68 (78.16)	0.727 <sup>b</sup>
Total cost of index admission, mean (sd)	309,380 (455,314)	353,907 (450,153)	354,996 (438,161)	0.222 <sup>c</sup>
Total cost of index year (mean)	392,935 (471,912)	434,570 (471,404)	437,545 (452,828)	0.347 <sup>c</sup>
Total cost of index admission diagnostic pathway, mean (sd)	15,783 (10,630)	15,778 (6,538)	20,390 (8,241)	< 0.001 <sup>c</sup>
Total cost of index year diagnostic pathway, mean (sd)	19,858 (11,147)	17,767 (6,245)	22,551 (8,336)	< 0.001 <sup>c</sup>
Total cost of index genetic tests,	9,370 (7,781)	10,412 (3,821)	12,347 (4,868)	< 0.001 <sup>c</sup>

mean (sd)				
Total cost of index year genetic tests, mean (sd)	11,693 (8,348)	10,928 (3,317)	12,835 (4,614)	< 0.001 <sup>c</sup>
<sup>a</sup> All costs reported in 2017 USD\$				
<sup>b</sup> One-way ANOVA; <sup>c</sup> Kruskal-Wallis H test				

Table S5. Costs by diagnosis <sup>a</sup>		
	No-ES Cohort	ES Cohort
<b>CHARGE syndrome</b>	n=4	n=2
Length of stay, index admission, mean (days)	91	84
Survival to 1 year, n (%)	3 (75.0)	2 (100)
Total cost of index admission, mean (sd)	431,360 (283,787)	317,590 (333,997)
Total cost of index year (mean)	456,497 (290,059)	400,245 (266,596)
Total cost of index admission diagnostic pathway, mean (sd)	8,142 (2,741)	15,266 (15,746)
Total cost of index year diagnostic pathway, mean (sd)	8,778 (2,141)	20,951 (8,782)
Total cost of index genetic tests, mean (sd)	2,983 (552)	7,739 (7,856)
Total cost of index year genetic tests, mean (sd)	2,983 (552)	11,517 (2,512)
<b>Noonan syndrome<sup>b</sup></b>	n=3	n=9
Length of stay, index admission, mean (days)	27	54
Survival to 1 year, n (%)	3 (100)	6 (66.7)
Total cost of index admission, mean (sd)	102,819 (120,924)	212,770 (198,847)
Total cost of index year (mean)	109,584 (121,021)	336,825 (301,821)
Total cost of index admission diagnostic pathway, mean (sd)	5,511 (4,989)	9,931 (5,083)
Total cost of index year diagnostic pathway, mean (sd)	5,568 (4,911)	16,276 (6,979)
Total cost of index genetic tests, mean (sd)	2,894 (2,481)	5,496 (4,583)
Total cost of index year genetic tests, mean (sd)	2,894 (2,481)	9,062 (4,890)
<b>Walker-Warburg syndrome</b>	n=1	n=1

Length of stay, index admission (days)	31	36
<b>Gaucher disease</b>	n=1	n=1
Length of stay, index admission (days)	4	29
ES, exome sequencing		
<sup>a</sup> All costs reported in 2017 USD\$		
<sup>b</sup> includes Neurofibromatosis-Noonan syndrome, and Noonan syndrome 1, 3, and 5 diagnoses		

## **CONCLUSION**

This project presents evidence, both through literature review and analysis of a newly assembled dataset through systematic collection and merging of data, on the impact of clinical ES on the cost of care for infants undergoing a diagnostic workup for suspected genetic disease. Uptake of clinical genomic sequencing has occurred quickly and without robust, systematic evidence regarding either outcomes or costs from comparative studies to inform its application. In the patient population studied herein, ES demonstrated important diagnostic utility for patients with monogenic disease, yet other genetic tests, especially chromosomal microarray, remain important diagnostic tools given the burden of chromosomal abnormalities in this population. As clinically applied over the first 5 years, ES does not appear cost-effective as a diagnostic tool for the broad population of newborns and infants with suspected genetic disease as compared to standard diagnostics. Further work is needed to develop outcome measures to capture utility of both diagnostic ES results and non-diagnostic ES results for clinicians, patients, and patients' families and to specify clinical guidelines for appropriate ES application.



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