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

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Purpose: Availability of clinical genomic sequencing (CGS) has generated questions about the value of 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 evaluation, is needed to demonstrate clinical utility and cost-effectiveness of CGS.

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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.[1] Clinical genomic sequencing (CGS), which includes genome sequencing (GS) and exome sequencing (ES), 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 ES 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.[2] 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.[3] 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 nonrapid), 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

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outcomes.[4, 5] 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.[6]

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,[7] which may be performed in addition to or in place of other nongenetic investigations. Although sequencing has typically been recommended for patients with nonspecific clinical features that may be associated with numerous underlying causes (even those that are not yet well established),[7, 8] 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.[9, 10] 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.[3, 11] While numerous methodological challenges exist for economic evaluations of genomic sequencing tests,[12] 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.[13] While diagnostic yield is an important outcome, it is only an 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.[14, 15] Determination of CGS's value for any specific clinically defined group of patients is further complicated by statistical uncertainty about outcomes (including diagnostic yield) due to small sample sizes, which can obstruct economic model development.[16]

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 with the gold standard dideoxy method have been explored.[17] 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 ES was shown.[18, 19]

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,[20] adapted for use in a scoping review as appropriate. CGS is defined to include GS 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 nonrapid 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.[21] 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, MA), 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 predetermined 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 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 postmortem 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.[22] 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 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.[23] 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, TX).

RESULTS

Study selection

The study selection process is summarized as a PRISMA flow diagram in Fig. 1. Database searches and a hand search yielded 3039 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[19, 24–153] and 40 (24%) were aggregate analyses[5, 6, 154–191]. Four studies had a primary objective of economic evaluation and also reported primary effectiveness data[153, 189–191]. The number of included articles increased by publication year. One article each year was included from

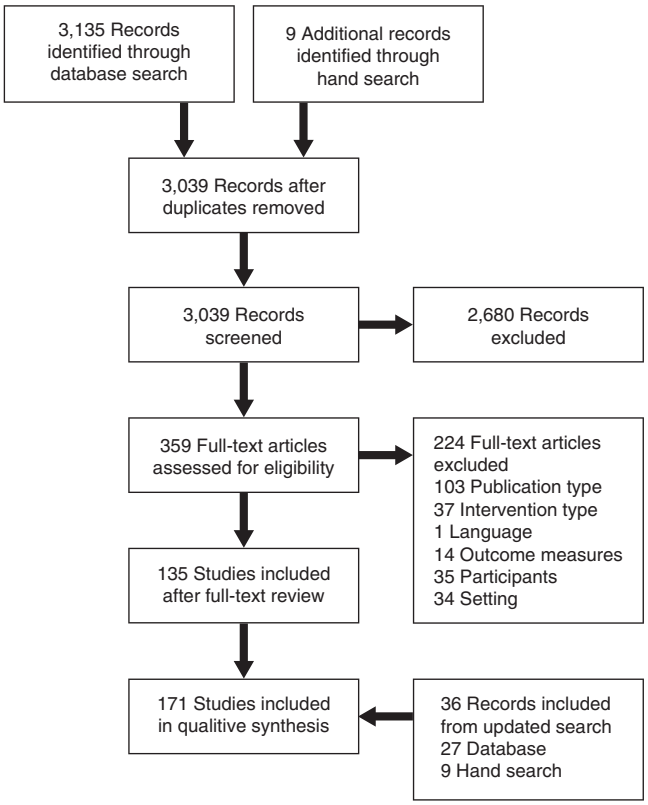

Fig. 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection

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 United States (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. Of 24 items on the CHEERS checklist recommended for reporting, the economic evaluation articles reported 7, 14, 18, and 17 items.

Syntheses of results

ES was used in 93% (159/171) of articles, GS in 6% (10/171), and a combination of ES and GS 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 nonspecific 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.[192] 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 to 100%, with a median of 33.2%. Other than 3 studies that reported 100% yield, the highest yield was 68.3%.[178] Beyond diagnostic yield, other health outcome measures of the downstream effect of sequencing on medical management were listed[5, 6, 165, 173–175, 178, 180, 182] or presented in a table[154, 159, 172] in 30% of large sample studies. Of the 12 studies that measured them, 8 studies[5, 6, 172–175, 178, 182] provided a definition of outcomes, including providing specific examples of the types of care changes included in each category.

Table 1 Summary of large sample studies

First author (year), country	Type of CGS	Study population	Overall diagnostic yield (%); subanalysis by test type or comparator yield (%)	Change in mgmt (%)[a]
Bick D (2017), USA[154]	GS	Suspected Mendelian disorder	3/22 (14); After reanalysis, 8/22 (36)	6/8 (75)
Bowling KM (2017), USA[155]	ES, GS	DD and/or ID	100/371 (27); Trio: 90/309 (29), Duo: 8/42 (19), Proband: 3/20 (15)	
Farwell KD (2015), USA[156]	ES	Consecutive samples sent to diagnostic lab	152/500 (30); Trio: 82/220 (37), Proband: 14/68 (21)	
Gauthier-Vasserot A (2017), France[157]	ES	Syndromic congenital neutropenia with ID	4/10 (40)	
Helbig KL (2016), USA[158]	ES	Consecutive samples sent to diagnostic lab	322/1131 (28)[b]	
Iglesias A (2014), USA[159]	ES	Consecutive patients in genetics center	37/115 (32)	24/37 (65)[b]
Lazaridis KN (2016), USA[160]	ES	Diagnostic odyssey	15/51 (29)	
Lee H (2014), USA[161]	ES	Consecutive patients referred to clinical lab	213/814 (26); Trio: 127/410 (31), Proband: 74/338 (22)	
Lionel AC (2017), Canada[162]	GS	Suspected genetic etiology	42/103 (41); Conventional genetic testing: 25/103 (24)	
Meng L (2017), USA[5]	ES	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[163]	ES	Consecutive CA and ID patients	128/416 (31) over 3 years with 2 reanalyses; yield per year ranged 22–27%	9/128 (7)
Need AC (2012), USA[164]	ES	ID/DD, CA, or facial dysmorphisms	6/12 (50)	
Nolan D (2015), USA[165]	ES	Neurology clinic	24/50 (48)	8/24 (33)[b]
Ream MA (2014), USA[166]	ES	Drug-resistant epilepsy	1/6 (17)	0/6 (0)
Romasko EJ (2017), USA[167]	ES	Suspected inherited platelet disorder	5/21 (24)	1/5 (20)
Rump P (2016), Netherlands[168]	ES	ID and microcephaly	11/38 (29)	
Sawyer SL (2015), Canada[169]	ES	Diagnostic odyssey	105/362 (29) families	6/105 (6) families
Shamriz O (2017), Israel[170]	ES	Malignant infantile osteopetrosis	6/6 (100)	2/6 (33)
Shashi V (2016), USA[171]	ES	Outpatient pediatric genetics clinic	24/93 (26)[c]	
Soden SE (2014), USA[172]	ES, rapid GS	Neurodevelopmental disorders	53/119 (45) patients, 45/100 (45) families; NICU/PICU: 11/15 (73) families by rapid GS; Ambulatory: 34/85 (40) families by ES (1 by GS after negative ES)	22/49 families (45)
Srivastava S (2014), USA[173]	ES	Neurodevelopmental disorders	32/78 (41)	32/32 (100)

Table 1 continued

First author (year), country	Type of CGS	Study population	Overall diagnostic yield (%); subanalysis by test type or comparator yield (%)	Change in mgmt (%)[a]
Stark Z (2016), Australia[174]	ES	Suspected monogenetic disorder	46/80 (58); Standard diagnostics: 11/80 (14)	15/46 (33)
Stavropoulos DJ (2016), Canada[175]	GS	Referred for CMA by clinical geneticists	34/100 (34) by GS; CMA + targeted gene sequencing: 13/100 (13), CMA alone: 8/100 (8)	32/34 (94)
Takeichi T (2013), Kuwait[176]	ES	Pediatric dermatology genetics clinic	7/7 (100)	7/7 (100)
Tammimies K (2015), Canada[177]	ES	Developmental pediatrics clinics	8/95 (8); CMA: 24/258 (9)	
Tarailo-Graovac M (2016), Canada[178]	ES	Potential ID with metabolic phenotype	28/41 (68)	18/41 (44)
Taylor RW (2014), UK[179]	ES	Suspected mitochondrial disease	28/53 (53)	0/28 (0)
Thevenon J (2016), France[180]	ES	ID and/or epileptic encephalopathy	14/43 (33); Familial: 6/9 (67)	2/14 (14)
Trujillano D (2017), Germany[181]	ES	Suspected Mendelian disorder	307/1000 (31)	
Valencia CA (2015), USA[182]	ES	Diagnostic odyssey	12/40 (30)	12/12 (100)
Vissers LE (2017), Netherlands[183]	ES	Nonacute; neurological symptoms with suspected genetic etiology	44/150 (29); Standard diagnostics: 11/150 (7)	
Willig LK (2015), USA[6]	Rapid GS	Critically ill; suspected monogenetic disorder	20/35 (57); Standard genetic testing: 3/32 (9)	13/20 (65)
Wortmann SB (2015), Netherlands[184]	ES	Suspected mitochondrial disease	42/109 (39); MD gene "virtual panel": 21/42 (50), outside gene panel (ES): 28/42 (67)	
Yang Y (2013), USA[185]	ES	Consecutive samples sent to diagnostic lab	62/250 (25)	
Yang Y (2014), USA[186]	ES	Consecutive samples sent to diagnostic lab	504/2000 (25)	
Yavarna T (2015), Qatar[187]	ES	Suspected Mendelian disease	89/149 (60)	
Zhang J (2016), Australia[188]	ES	Hematological disorders with suspected genetic etiology	6/6 (100)	

CGS clinical genomic sequencing, *ES* exome sequencing, *GS* genome sequencing, *CMA* chromosomal microarray, *ID* intellectual disability, *DD* developmental delay, *CA* congenital anomaly,*NICU* neonatal intensive care unit, *PICU* pediatric intensive care unit

[a]Change in medical management overall (any change considered by the study's authors)

[b]Author's calculation based on presented data

[c]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 (in USD)[a]
Joshi (2016)[153] USA / hospital (not stated)	Descriptive; trio ES; standard diagnostics	Epilepsy center; $n = 4$ (including 2 siblings)	Total charges for standard diagnostics range $9,015–$35,483; charge for trio ES $6,100 / not calculated
Monroe (2016)[189] Netherlands / hospital system	Scenario analysis; trio ES; 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, ES 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, ES to replace genetic tests would save $5,669 on average. / not calculated
Stark (2017)[190] Australia / hospital system	CEA; proband ES; standard diagnostics	NICU, PICU, other inpatient, and outpatient; $n = 40$	Avg. cost per Dx, traditional diagnostics: $21,099, ES: $3,937 / ES as a first-tier diagnostic test: savings of $1,702; ES to replace some diagnostic tests: $2,045; ES after all other diagnostic tests: $6,327
Tan (2017)[191] Australia / health care system (not stated)	CEA; proband ES; standard diagnostics	Ambulatory outpatient clinics; $n = 44$	Avg. diagnostic odyssey 6 years, 19 tests, cost of $7,509. Cost per patient of ES at initial genetics appointment $3,933. / ES at initial tertiary clinical presentation: savings of $6,840; ES at initial genetics consult: savings of $4,143; ES after standard diagnostics: $4,371

CGS clinical genomic sequencing, *Dx* diagnosis, *ES* exome sequencing, *CEA* cost-effectiveness analysis, *NICU* neonatal intensive care unit, *PICU* pediatric intensive care unit

[a]All costs reported.

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. Nondiagnostic 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),[5, 6, 161, 174, 185] highlighted that CGS may shorten the time and cost involved in the diagnostic odyssey or sequential single-gene testing (6 articles),[5, 156, 159, 162, 168, 170] or provided an illustrative example or summary statistics on the number or cost of negative diagnostic tests performed prior to CGS (10 articles),[156, 162, 165, 168, 171, 172, 175, 182, 183, 185] 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.[6, 162, 174, 175, 183] 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 ES 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 ES found that first-tier ES was associated with an incremental cost savings of US $1702 per additional diagnosis, and when ES was performed after standard diagnostics, the incremental cost per additional diagnosis was US $6327.[190] Another study estimated incremental savings of US $6840 per diagnosis when ES was performed at the initial tertiary clinical visit and incremental cost of US $4371 when ES was used after

standard diagnostic investigations.[191] These results underline the role of timing and number of other nondiagnostic investigations performed in whether incremental diagnoses via ES 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 nondiagnostic 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, 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. ES 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 GS and ES 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.

ELECTRONIC SUPPLEMENTARY MATERIAL

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