# Resources on Whole Genome Sequencing

The Genomic Answers for Children's Health (GACH) Alliance has put together several compilations of studies and resources on whole genome sequencing (WGS). This document includes resources and studies on a variety of areas, including:

- Third-Party Policy Guidance on rapid Whole Genome Sequencing: a study that provides policy recommendations for a variant of WGS.
- Medicaid Coverage of rapid Whole Genome Sequencing: legislation in various states that pertains to Medicaid coverage of WGS.
- Commercial Coverage of rapid Whole Genome Sequencing: resources on the ways in which commercial payers provide coverage for WGS.
- Additional Peer-Reviewed Literature

## About the GACH Alliance

The GACH Alliance is a diverse group of health care stakeholders committed to expanding access to WGS and helping to end the diagnostic odyssey. Our mission is to ensure that any child with a rare disease or suspected genetic disorder who is enrolled in Medicaid has timely access to genomic sequencing, especially whole genome sequencing. We also seek to ensure that patients and their families have the opportunity to share the sequencing results with medical researchers, to help aid in the discovery and development of new treatments for rare diseases.

To learn more about the Alliance, contact Josh Trent at josh.trent@leavittpartners.com, Clay Alspach at clay.alspach@leavittpartners.com, Melissa Pfaff at melissa.pfaff@leavittpartners.com, or Tanner Fliss at tanner.fliss@leavittpartners.com. Visit our website at gachalliance.org.

# Third-Party Policy Guidance on rapid Whole Genome Sequencing

**1.Patient-centered Laboratory Utilization Guideline Services. Seattle Children's Hospital. Rapid Genome Sequencing. 2022 June.** https://www.schplugs.org/wp-content/uploads/Rapid-Genome-Sequencing-Policy_June-2022-FINAL.pdf

**Background:** "Rapid genome sequencing (rGS) involves sequencing of coding and noncoding regions of the nuclear and mitochondrial genome. Rapid analysis has an average turnaround time of less than 14 days, and
typically less than 7 days. rGS has been proposed for diagnostic use in acutely-ill children who present
with complex phenotypes suspicious for a rare genetic condition, who cannot be diagnosed by standard
clinical evaluation rapidly, or when features suggest a broad differential diagnosis that would require
evaluation by multiple genetic tests. When used as a first-tier test in the acutely-ill child, rGS can identify a genetic diagnosis efficiently, which has clinical utility in changing acute medical or surgical
management and improving outcomes.
Identifying a molecularly confirmed diagnosis in a timely manner for acutely-ill children witha rare
genetic condition can have a variety of health outcomes1-17
including but not limited to:

- guiding prognosis and improving clinical decision-making via
  - application of specific treatments, as well as withholding of contraindicated treatments for certain rare genetic conditions
  - planning or avoidance of surgical interventions
  - surveillance for comorbidities
  - initiation of palliative care
- reducing the psychological and financial impact of diagnostic uncertainty and the diagnostic odyssey (e.g., eliminating lower-yield testing and additional screening testing that may later be proven unnecessary once a diagnosis is achieved)
- allowing for more rapid molecular diagnosis than a sequential genetic testing approach
- informing genetic counseling for other living relatives (i.e., siblings), as well as recurrence risk counseling and prenatal diagnosis options for the family" https://www.schplugs.org/wp-content/uploads/Rapid-Genome-Sequencing-Policy_June-2022-FINAL.pdf

Genomic Answers FOR Children's Health ALLIANCE

## Medicaid Coverage of rapid Whole Genome Sequencing

1. Arizona: AHCCCS Reimbursement for Rapid Whole Genome Sequencing
2. California: Legislation, CA AB133 Health Trailer Bill
3. Florida: Laboratory Services Coverage Policy Agency for Health Care Administration
4. Georgia: January 2024 Update Laboratory Services
5. Louisiana: Legislation, LA SB154 & Clinical Policy: Rapid Whole Genome Sequencing of Critically Ill Infants
6. Maryland: Whole Genome Sequencing Clinical Criteria
7. Michigan: Laboratory Policy MSA 21-33: Coverage of Rapid Whole Genome Sequencing (rWGS) Testing
8. Minnesota: Laboratory and Pathology Services
9. Oregon: Ancillary/Diagnostic Guideline Notes for the Prioritized List of Health Services
10. Utah: Physician Services Manual Section 8-12:10.4 Next Generation Sequencing (NGS)

## Commercial Coverage of rapid Whole Genome Sequencing

- See landing page: https://radygenomics.org/clinical-genome-services/payer-policy-advocacy/.

## Additional Peer-Reviewed Literature

**1.Mestek-Boukhibar L, Clement E, Jones WD, et al. Rapid Paediatric Sequencing (RaPS): comprehensive real-life workflow for rapid diagnosis of critically ill children. J Med Genet. 2018 Nov;55(11):721-728. PMID: 30049826**

- **Abstract:** "Background: Rare genetic conditions are frequent risk factors for, or direct causes of, paediatric intensive care unit (PICU) admission. Such conditions are frequently suspected but unidentified at PICU admission. Compassionate and effective care is greatly assisted by definitive diagnostic information. There is therefore a need to provide a rapid genetic diagnosis to inform clinical management.To date, whole genome sequencing (WGS) approaches have proved successful in diagnosing a proportion of children with rare diseases, but results may take months to report. Our aim was to develop an end-to-end workflow for the use of rapid WGS for diagnosis in critically ill children in a UK National Health Service (NHS) diagnostic setting.
- **Methods**: We sought to establish a multidisciplinary Rapid Paediatric Sequencing team for case selection, trio WGS, rapid bioinformatics sequence analysis and a phased analysis and reporting system to prioritise genes with a high likelihood of being causal.
- **Results**: Trio WGS in 24 critically ill children led to a molecular diagnosis in 10 (42%) through the identification of causative genetic variants. In 3 of these 10 individuals (30%), the diagnostic result had an immediate impact on the individual's clinical management. For the last 14 trios, the shortest time taken to reach a provisional diagnosis was 4 days (median 8.5 days).
- **Conclusion**: Rapid WGS can be used to diagnose and inform management of critically ill children within the constraints of an NHS clinical diagnostic setting. We provide a robust workflow that will inform and facilitate the rollout of rapid genome sequencing in the NHS and other healthcare systems globally."
  https://pubmed.ncbi.nlm.nih.gov/30049826/

**2.French CE, Delon I, Dolling H, et al. Whole genome sequencing reveals that genetic conditions are frequent in intensively ill children. Intensive Care Med. 2019 May;45(5):627- 636. PMID: 30847515**

- **Abstract:** "Purpose: With growing evidence that rare single gene disorders present in the neonatal period, there is a need for rapid, systematic, and comprehensive genomic diagnoses in ICUs to assist acute and long-term clinical decisions. This study aimed to identify genetic conditions in neonatal (NICU) and paediatric (PICU) intensive care populations.
- **Methods**: We performed trio whole genome sequence (WGS) analysis on a prospective cohort of families recruited in NICU and PICU at a single site in the UK. We developed a research pipeline in collaboration with the National Health Service to deliver validated pertinent pathogenic findings within 2-3 weeks of recruitment.
- **Results**: A total of 195 families had whole genome analysis performed (567 samples) and 21% received a molecular diagnosis for the underlying genetic condition in the child.

The phenotypic description of the child was a poor predictor of the gene identified in 90% of cases, arguing for gene agnostic testing in NICU/PICU. The diagnosis affected clinical management in more than 65% of cases (83% in neonates) including modification of treatments and care pathways and/or informing palliative care decisions. A 2-3 week turnaround was sufficient to impact most clinical decision-making.

- **Conclusions**: The use of WGS in intensively ill children is acceptable and trio analysis facilitates diagnoses. A gene agnostic approach was effective in identifying an underlying genetic condition, with phenotypes and symptomatology being primarily used for data interpretation rather than gene selection. WGS analysis has the potential to be a first-line diagnostic tool for a subset of intensively ill children." https://pubmed.ncbi.nlm.nih.gov/30847515/

**3.Bertoli-Avella AM, Beetz C, Ameziane N, et al. Successful application of genome sequencing in a diagnostic setting: 1007 index cases from a clinically heterogeneous cohort. Eur J Hum Genet. 2020.**

- **Abstract:** "Despite clear technical superiority of genome sequencing (GS) over other diagnostic methods such as exome sequencing (ES), few studies are available regarding the advantages of its clinical application. We analyzed 1007 consecutive index cases for whom GS was performed in a diagnostic setting over a 2-year period. We reported pathogenic and likely pathogenic (P/LP) variants that explain the patients' phenotype in 212 of the 1007 cases (21.1%). In 245 additional cases (24.3%), a variant of unknown significance (VUS) related to the phenotype was reported. We especially investigated patients which had had ES with no genetic diagnosis (n = 358). For this group, GS diagnostic yield was 14.5% (52 patients with P/LP out of 358). GS should be especially indicated for ES-negative cases since up to 29.6% of them could benefit from GS testing (14.5% with P/LP, n = 52 and 15.1% with VUS, n = 54). Genetic diagnoses in most of the ES-negative/GS-positive cases were determined by technical superiority of GS, i.e., access to noncoding regions and more uniform coverage. Importantly, we reported 79 noncoding variants, of which, 41 variants were classified as P/LP. Interpretation of noncoding variants remains challenging, and in many cases, complementary methods based on direct enzyme assessment, biomarker testing and RNA analysis are needed for variant classification and diagnosis. We present the largest cohort of patients with GS performed in a clinical setting to date. The results of this study should direct the decision for GS as standard second-line, or even first-line stand-alone test." https://pubmed.ncbi.nlm.nih.gov/32860008/

**4.Bick D, Fraser PC, Gutzeit MF, et al. Successful Application of Whole Genome Sequencing in a Medical Genetics Clinic. J Pediatr Genet. 2017 Jun;6(2):61-76. PMID: 28496993**

- **Abstract:** "A pilot program was initiated using whole genome sequencing (WGS) to diagnose suspected genetic disorders in the Genetics Clinic at Children's Hospital of Wisconsin. Twenty-two patients underwent WGS between 2010 and 2013. Initially, we obtained a 14% (3/22) diagnosis rate over 2 years; with subsequent reanalysis, this increased to 36% (8/22). Disease causing variants were identified in SKIV2L, CECR1, DGKE, PYCR2, RYR1, PDGFRB, EFTUD2, and BCS1L. In 75% (6/8) of diagnosed

cases, the diagnosis affected treatment and/or medical surveillance. Additionally, one case demonstrated a homozygous A18V variant in VLDLR that appears to be associated with a previously undescribed phenotype." https://pubmed.ncbi.nlm.nih.gov/28496993/

**5.Bick D, Jones M, Taylor SL, Taft RJ, Belmont J. Case for genome sequencing in infants and children with rare, undiagnosed or genetic diseases. J Med Genet. 2019;56(12):783-791. doi:10.1136/jmedgenet-2019-106111**

- **Abstract:** "Up to 350 million people worldwide suffer from a rare disease, and while the individual diseases are rare, in aggregate they represent a substantial challenge to global health systems. The majority of rare disorders are genetic in origin, with children under the age of five disproportionately affected. As these conditions are difficult to identify clinically, genetic and genomic testing have become the backbone of diagnostic testing in this population. In the last 10 years, next-generation sequencing technologies have enabled testing of multiple disease genes simultaneously, ranging from targeted gene panels to exome sequencing (ES) and genome sequencing (GS). GS is quickly becoming a practical first-tier test, as cost decreases and performance improves. A growing number of studies demonstrate that GS can detect an unparalleled range of pathogenic abnormalities in a single laboratory workflow. GS has the potential to deliver unbiased, rapid and accurate molecular diagnoses to patients across diverse clinical indications and complex presentations. In this paper, we discuss clinical indications for testing and historical testing paradigms. Evidence supporting GS as a diagnostic tool is supported by superior genomic coverage, types of pathogenic variants detected, simpler laboratory workflow enabling shorter turnaround times, diagnostic and reanalysis yield, and impact on healthcare." https://pubmed.ncbi.nlm.nih.gov/31023718/

**6.Kingsmore SF, Cakici JA, Clark MM, et al. A Randomized, Controlled Trial of the Analytic and Diagnostic Performance of Singleton and Trio, Rapid Genome and Exome Sequencing in Ill Infants. Am J Hum Genet. 2019 Oct 3;105(4):719-733. PMID: 31564432**

- **Abstract:** "The second Newborn Sequencing in Genomic Medicine and Public Health study was a randomized, controlled trial of the effectiveness of rapid whole-genome or -exome sequencing (rWGS or rWES, respectively) in seriously ill infants with diseases of unknown etiology. Here we report comparisons of analytic and diagnostic performance. Of 1,248 ill inpatient infants, 578 (46%) had diseases of unknown etiology. 213 infants (37% of those eligible) were enrolled within 96 h of admission. 24 infants (11%) were very ill and received ultra-rapid whole-genome sequencing (urWGS). The remaining infants were randomized, 95 to rWES and 94 to rWGS. The analytic performance of rWGS was superior to rWES, including variants likely to affect protein function, and ClinVar pathogenic/likely pathogenic variants ($p < 0.0001$). The diagnostic performance of rWGS and rWES were similar (18 diagnoses in 94 infants [19%] versus 19 diagnoses in 95 infants [20%], respectively), as was time to result (median 11.0 versus 11.2 days, respectively). However, the proportion diagnosed by urWGS (11 of 24 [46%]) was higher than rWES/rWGS ($p = 0.004$) and time to result was less (median 4.6 days, $p < 0.0001$). The incremental diagnostic yield of reflexing to trio after negative proband analysis was 0.7% (1 of 147). In conclusion, rapid genomic sequencing can be performed as a first-tier diagnostic test in inpatient infants. urWGS had the shortest time to result, which was important in unstable infants, and those in whom a genetic diagnosis was likely to impact

immediate management. Further comparison of urWGS and rWES is warranted because genomic technologies and knowledge of variant pathogenicity are evolving rapidly." https://pubmed.ncbi.nlm.nih.gov/31564432/

**7.Dimmock DP, Clark MM, Gaughran M, et al. An RCT of Rapid Genomic Sequencing among Seriously Ill Infants Results in High Clinical Utility, Changes in Management, and Low Perceived Harm. Am J Hum Genet. 2020;107(5):942-952. doi:10.1016/j.ajhg.2020.10.003**

- **Abstract:** "The second Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT2) study was a randomized, controlled trial of rapid whole-genome sequencing (rWGS) or rapid whole-exome sequencing (rWES) in infants with diseases of unknown etiology in intensive care units (ICUs). Gravely ill infants were not randomized and received ultra-rapid whole-genome sequencing (urWGS). Herein we report results of clinician surveys of the clinical utility of rapid genomic sequencing (RGS). The primary end-point-clinician perception that RGS was useful- was met for 154 (77%) of 201 infants. Both positive and negative tests were rated as having clinical utility (42 of 45 [93%] and 112 of 156 [72%], respectively). Physicians reported that RGS changed clinical management in 57 (28%) infants, particularly in those receiving urWGS ($p = 0.0001$) and positive tests ($p < 0.00001$). Outcomes of 32 (15%) infants were perceived to be changed by RGS. Positive tests changed outcomes more frequently than negative tests ($p < 0.00001$). In logistic regression models, the likelihood that RGS was perceived as useful increased 6.7-fold when associated with changes in management (95% CI 1.8-43.3). Changes in management were 10.1-fold more likely when results were positive (95% CI 4.7-22.4) and turnaround time was shorter (odds ratio 0.92, 95% CI 0.85-0.99). RGS seldom led to clinician-perceived confusion or distress among families (6 of 207 [3%]). In summary, clinicians perceived high clinical utility and low likelihood of harm with first-tier RGS of infants in ICUs with diseases of unknown etiology. RGS was perceived as beneficial irrespective of whether results were positive or negative." https://pubmed.ncbi.nlm.nih.gov/33157007/

**8.Meienberg J, Bruggmann R, et al. Clinical sequencing: is WGS the better WES? Hum Genet. 2016; 135: 359–362.PMID: 26742503**

- **Abstract:** "Current clinical next-generation sequencing is done by using gene panels and exome analysis, both of which involve selective capturing of target regions. However, capturing has limitations in sufficiently covering coding exons, especially GC-rich regions. We compared whole exome sequencing (WES) with the most recent PCR-free whole genome sequencing (WGS), showing that only the latter is able to provide hitherto unprecedented complete coverage of the coding region of the genome. Thus, from a clinical/technical point of view, WGS is the better WES so that capturing is no longer necessary for the most comprehensive genomic testing of Mendelian disorders." https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4757617/

**9.Wang H, Lu Y, Dong X, et al. Optimized trio genome sequencing (OTGS) as a first-tier genetic test in critically ill infants: practice in China. Hum Genet. 2020 Apr;139(4):473-482. PMID: 31965297**

- **Abstract:** “Genome sequencing is used to make genetic diagnoses in critically ill infants with rapid turnaround time (TAT). Herein, to delineate the value of a genetic diagnosis, we provide the results from 130 pediatric patients in a large, comprehensive children's hospital in China. This study was performed using an optimized trio genome sequencing (OTGS) test. The sequencing depth for patients was 40-50 × and for their parents, it was 8-10 × . Patients from the pediatric or neonatal intensive care unit (PICU/NICU) with complicated clinical features were enrolled between June 2018 and December 2018, each with a phenotype suggesting an underlying genetic disorder. OTGS testing identified pathogenic variants in 62 of 130 individuals, resulting in a diagnosis rate of 47.7%. The TAT varied from 72 to 120 h, with an average of 94 h and a median of 90 h. Of the 62 infants with diagnoses, 48 (77.4%) had pathogenic single-nucleotide variants (SNVs), 12 (19.4%) had pathogenic copy number variations (CNVs) or structure variants (SVs), and 2 (3.2%) had small deletions in one allele plus pathogenic variants in another allele of autosomal recessive genes. Therapeutic strategies for 48.4% (30/62) of the diagnosed patients were modified and included transplantation, dietary recommendations, or change of drugs, which avoided morbidity and improved prognosis. This study provided high-capacity OTGS testing in detecting SNVs and chromosomal abnormalities with fast response, higher diagnostic yield, and lower cost. OTGS demonstrates the potential to be the first-tier of genetic testing used in critically ill infants in developing countries.” https://pubmed.ncbi.nlm.nih.gov/31965297/

**10.Wu B, Kang W, Wang Y, et al. Application of Full-Spectrum Rapid Clinical Genome Sequencing Improves Diagnostic Rate and Clinical Outcomes in Critically Ill Infants in the China Neonatal Genomes Project. Crit Care Med. 2021 Oct 1;49(10):1674-1683. PMID: 33935161**

- **Abstract:** “Objectives: To determine the diagnostic and clinical utility of trio-rapid genome sequencing in critically ill infants.
- **Design**: In this prospective study, samples from critically ill infants were analyzed using both proband-only clinical exome sequencing and trio-rapid genome sequencing (proband and biological parents). The study occurred between April 2019 and December 2019.
- **Setting**: Thirteen member hospitals of the China Neonatal Genomes Project spanning 10 provinces were involved.
- **Participants**: Critically ill infants (n = 202), from birth up until 13 months of life were enrolled based on eligibility criteria (e.g., CNS anomaly, complex congenital heart disease, evidence of metabolic disease, recurrent severe infection, suspected immune deficiency, and multiple malformations).
- **Interventions**: None.
- **Measurements and main results**: Of the 202 participants, neuromuscular (45%), respiratory (22%), and immunologic/infectious (18%) were the most commonly observed phenotypes. The diagnostic yield of trio-rapid genome sequencing was higher than that of proband-only clinical exome sequencing (36.6% [95% CI, 30.1-43.7%] vs 20.3% [95% CI, 15.1-26.6%], respectively; p = 0.0004), and the average turnaround time for trio-rapid

genome sequencing (median: 7 d) was faster than that of proband-only clinical exome sequencing (median: 20 d) (p < 2.2 × 10-16). The metagenomic analysis identified pathogenic or likely pathogenic microbes in six infants with symptoms of sepsis, and these results guided the antibiotic treatment strategy. Sixteen infants (21.6%) experienced a change in clinical management following trio-rapid genome sequencing diagnosis, and 24 infants (32.4%) were referred to a new subspecialist.

- **Conclusions**: Trio-rapid genome sequencing provided higher diagnostic yield in a shorter period of time in this cohort of critically ill infants compared with proband-only clinical exome sequencing. Precise and fast molecular diagnosis can alter medical management and positively impact patient outcomes." https://pubmed.ncbi.nlm.nih.gov/33935161/

**11.Sweeney NM, Nahas SA, Chowdhury S, et al. Rapid whole genome sequencing impacts care and resource utilization in infants with congenital heart disease. NPJ Genom Med. Apr 22 2021;6(1):29. PMID: 33888711**

- **Abstract:** "Congenital heart disease (CHD) is the most common congenital anomaly and a major cause of infant morbidity and mortality. While morbidity and mortality are highest in infants with underlying genetic conditions, molecular diagnoses are ascertained in only ~20% of cases using widely adopted genetic tests. Furthermore, cost of care for children and adults with CHD has increased dramatically. Rapid whole genome sequencing (rWGS) of newborns in intensive care units with suspected genetic diseases has been associated with increased rate of diagnosis and a net reduction in cost of care. In this study, we explored whether the clinical utility of rWGS extends to critically ill infants with structural CHD through a retrospective review of rWGS study data obtained from inpatient infants < 1 year with structural CHD at a regional children's hospital. rWGS diagnosed genetic disease in 46% of the enrolled infants. Moreover, genetic disease was identified five times more frequently with rWGS than microarray ± gene panel testing in 21 of these infants (rWGS diagnosed 43% versus 10% with microarray ± gene panels, p = 0.02). Molecular diagnoses ranged from syndromes affecting multiple organ systems to disorders limited to the cardiovascular system. The average daily hospital spending was lower in the time period post blood collection for rWGS compared to prior (p = 0.003) and further decreased after rWGS results (p = 0.000). The cost was not prohibitive to rWGS implementation in the care of this cohort of infants. rWGS provided timely actionable information that impacted care and there was evidence of decreased hospital spending around rWGS implementation." https://pubmed.ncbi.nlm.nih.gov/33888711/

**12.Bick D, Bick SL, Dimmock DP, et al. An online compendium of treatable genetic disorders. Am J Med Genet C Semin Med Genet. 2021 Mar;187(1):48-54. PMID: 33350578**

- **Abstract:** "More than 4,000 genes have been associated with recognizable Mendelian/monogenic diseases. When faced with a new diagnosis of a rare genetic disorder, health care providers increasingly turn to internet resources for information to understand the disease and direct care. Unfortunately, it can be challenging to find information concerning treatment for rare diseases as key details are scattered across a number of authoritative websites and numerous journal articles. The website and

associated mobile device application described in this article begin to address this challenge by providing a convenient, readily available starting point to find treatment information. The site, Rx-genes.com (https://www.rx-genes.com/), is focused on those conditions where the treatment is directed against the mechanism of the disease and thereby alters the natural history of the disease. The website currently contains 633 disease entries that include references to disease information and treatment guidance, a brief summary of treatments, the inheritance pattern, a disease frequency (if known), nonmolecular confirmatory testing (if available), and a link to experimental treatments. Existing entries are continuously updated, and new entries are added as novel treatments appear in the literature." https://pubmed.ncbi.nlm.nih.gov/33350578/

**13.Gilissen C, Hehir-Kwa JY, Thung DT, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature. 2014 Jul 17;511(7509):344-7. PMID: 24896178**

- **Abstract:** "Severe intellectual disability (ID) occurs in 0.5% of newborns and is thought to be largely genetic in origin. The extensive genetic heterogeneity of this disorder requires a genome-wide detection of all types of genetic variation. Microarray studies and, more recently, exome sequencing have demonstrated the importance of de novo copy number variations (CNVs) and single-nucleotide variations (SNVs) in ID, but the majority of cases remain undiagnosed. Here we applied whole-genome sequencing to 50 patients with severe ID and their unaffected parents. All patients included had not received a molecular diagnosis after extensive genetic prescreening, including microarray-based CNV studies and exome sequencing. Notwithstanding this prescreening, 84 de novo SNVs affecting the coding region were identified, which showed a statistically significant enrichment of loss-of-function mutations as well as an enrichment for genes previously implicated in ID-related disorders. In addition, we identified eight de novo CNVs, including single-exon and intra-exonic deletions, as well as interchromosomal duplications. These CNVs affected known ID genes more frequently than expected. On the basis of diagnostic interpretation of all de novo variants, a conclusive genetic diagnosis was reached in 20 patients. Together with one compound heterozygous CNV causing disease in a recessive mode, this results in a diagnostic yield of 42% in this extensively studied cohort, and 62% as a cumulative estimate in an unselected cohort. These results suggest that de novo SNVs and CNVs affecting the coding region are a major cause of severe ID. Genome sequencing can be applied as a single genetic test to reliably identify and characterize the comprehensive spectrum of genetic variation, providing a genetic diagnosis in the majority of patients with severe ID." https://pubmed.ncbi.nlm.nih.gov/24896178/

**14.Marshall CR, Chowdhury S, Taft RJ, et al. Best practices for the analytical validation of clinical whole-genome sequencing intended for the diagnosis of germline disease. NPJ Genom Med. 2020 Oct 23;5:47. PMID: 33110627**

- **Abstract:** "Whole-genome sequencing (WGS) has shown promise in becoming a first-tier diagnostic test for patients with rare genetic disorders; however, standards addressing the definition and deployment practice of a best-in-class test are lacking. To address these gaps, the Medical Genome Initiative, a consortium of leading healthcare and research organizations in the US and Canada, was formed to expand access to

high-quality clinical WGS by publishing best practices. Here, we present consensus recommendations on clinical WGS analytical validation for the diagnosis of individuals with suspected germline disease with a focus on test development, upfront considerations for test design, test validation practices, and metrics to monitor test performance. This work also provides insight into the current state of WGS testing at each member institution, including the utilization of reference and other standards across sites. Importantly, members of this initiative strongly believe that clinical WGS is an appropriate first-tier test for patients with rare genetic disorders, and at minimum is ready to replace chromosomal microarray analysis and whole-exome sequencing. The recommendations presented here should reduce the burden on laboratories introducing WGS into clinical practice, and support safe and effective WGS testing for diagnosis of germline disease." https://pubmed.ncbi.nlm.nih.gov/33110627/

**15.Lionel AC, Costain G, Monfared N, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. Genet Med. 2018 Apr;20(4):435-443. PMID: 28771251**

- **Abstract:** "PurposeGenetic testing is an integral diagnostic component of pediatric medicine. Standard of care is often a time-consuming stepwise approach involving chromosomal microarray analysis and targeted gene sequencing panels, which can be costly and inconclusive. Whole-genome sequencing (WGS) provides a comprehensive testing platform that has the potential to streamline genetic assessments, but there are limited comparative data to guide its clinical use.MethodsWe prospectively recruited 103 patients from pediatric non-genetic subspecialty clinics, each with a clinical phenotype suggestive of an underlying genetic disorder, and compared the diagnostic yield and coverage of WGS with those of conventional genetic testing.ResultsWGS identified diagnostic variants in 41% of individuals, representing a significant increase over conventional testing results (24%; P = 0.01). Genes clinically sequenced in the cohort (n = 1,226) were well covered by WGS, with a median exonic coverage of 40 × ±8 × (mean ±SD). All the molecular diagnoses made by conventional methods were captured by WGS. The 18 new diagnoses made with WGS included structural and non-exonic sequence variants not detectable with whole-exome sequencing, and confirmed recent disease associations with the genes PIGG, RNU4ATAC, TRIO, and UNC13A.ConclusionWGS as a primary clinical test provided a higher diagnostic yield than conventional genetic testing in a clinically heterogeneous cohort." https://pubmed.ncbi.nlm.nih.gov/28771251/

**16.Gross A, Subramanian S, et al. Copy-number variants in clinical genome sequencing: deployment and interpretation for rare and undiagnosed disease. Genet Med. May 2018; 21(5):1121-1130. PMID 30293986**

- **Abstract:** "Purpose: Current diagnostic testing for genetic disorders involves serial use of specialized assays spanning multiple technologies. In principle, genome sequencing (GS) can detect all genomic pathogenic variant types on a single platform. Here we evaluate copy-number variant (CNV) calling as part of a clinically accredited GS test.
- **Methods**: We performed analytical validation of CNV calling on 17 reference samples, compared the sensitivity of GS-based variants with those from a clinical microarray, and

set a bound on precision using orthogonal technologies. We developed a protocol for family-based analysis of GS-based CNV calls, and deployed this across a clinical cohort of 79 rare and undiagnosed cases.

- **Results**: We found that CNV calls from GS are at least as sensitive as those from microarrays, while only creating a modest increase in the number of variants interpreted (~10 CNVs per case). We identified clinically significant CNVs in 15% of the first 79 cases analyzed, all of which were confirmed by an orthogonal approach. The pipeline also enabled discovery of a uniparental disomy (UPD) and a 50% mosaic trisomy 14. Directed analysis of select CNVs enabled breakpoint level resolution of genomic rearrangements and phasing of de novo CNVs.
- **Conclusion**: Robust identification of CNVs by GS is possible within a clinical testing environment." https://pubmed.ncbi.nlm.nih.gov/30293986/

**17.Malinowski et al. Systematic evidence-based review: outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability. https://pubmed.ncbi.nlm.nih.gov/32203227/**

- **Abstract:** "Purpose: Exome and genome sequencing (ES/GS) are performed frequently in patients with congenital anomalies, developmental delay, or intellectual disability (CA/DD/ID), but the impact of results from ES/GS on clinical management and patient outcomes is not well characterized. A systematic evidence review (SER) can support future evidence-based guideline development for use of ES/GS in this patient population.
- **Methods**: We undertook an SER to identify primary literature from January 2007 to March 2019 describing health, clinical, reproductive, and psychosocial outcomes resulting from ES/GS in patients with CA/DD/ID. A narrative synthesis of results was performed.
- **Results**: We retrieved 2654 publications for full-text review from 7178 articles. Only 167 articles met our inclusion criteria, and these were primarily case reports or small case series of fewer than 20 patients. The most frequently reported outcomes from ES/GS were changes to clinical management or reproductive decision-making. Two studies reported on the reduction of mortality or morbidity or impact on quality of life following ES/GS.
- **Conclusion**: There is evidence that ES/GS for patients with CA/DD/ID informs clinical and reproductive decision-making, which could lead to improved outcomes for patients and their family members. Further research is needed to generate evidence regarding health outcomes to inform robust guidelines regarding ES/GS in the care of patients with CA/DD/ID." https://pubmed.ncbi.nlm.nih.gov/32203227/

**18.Guo F, et al. Evidence from 2100 index cases supports genome sequencing as a first-tier genetic test. Genet Med. 2024 Jan;26(1):100995. https://pubmed.ncbi.nlm.nih.gov/37838930/**

- **Abstract:** "Purpose: Genome sequencing (GS) is one of the most comprehensive assays that interrogate single-nucleotide variants, copy number variants, mitochondrial

variants, repeat expansions, and structural variants in a single assay. Despite the clear technical superiority, the full clinical utility of GS has yet to be determined.

- **Methods**: We systematically evaluated 2100 clinical GS index cases performed in our laboratory to explore the diagnostic yield of GS as first-tier and as follow-up testing.
- **Results**: The overall diagnostic yield was 28% (585/2100). The diagnostic yield for GS as the first-tier test was 26% (294/1146). Among cases with prior non-diagnostic genetic tests, GS provided a diagnosis for 27% (247/910) of cases, including 56 cases with prior exome sequencing (ES). Although re-analysis of previous ES might have resolved the diagnosis in 29 cases, diagnoses for 27 cases would have been missed because of the technical inferiority of ES. Moreover, GS further disclosed additional genetic etiology in 3 out of 44 cases with existing partial diagnosis.
- **Conclusion**: We present the largest-to-date GS data set of a clinically heterogeneous cohort from a single clinical laboratory. Our data demonstrate that GS should be considered as the first-tier genetic test that has the potential to shorten the diagnostic odyssey." https://pubmed.ncbi.nlm.nih.gov/37838930/

**19.Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. Nat Genet. 2015 Jul;47(7):717-726. PMID: 25985138**

- **Abstract:** "To assess factors influencing the success of whole-genome sequencing for mainstream clinical diagnosis, we sequenced 217 individuals from 156 independent cases or families across a broad spectrum of disorders in whom previous screening had identified no pathogenic variants. We quantified the number of candidate variants identified using different strategies for variant calling, filtering, annotation and prioritization. We found that jointly calling variants across samples, filtering against both local and external databases, deploying multiple annotation tools and using familial transmission above biological plausibility contributed to accuracy. Overall, we identified disease-causing variants in 21% of cases, with the proportion increasing to 34% (23/68) for mendelian disorders and 57% (8/14) in family trios. We also discovered 32 potentially clinically actionable variants in 18 genes unrelated to the referral disorder, although only 4 were ultimately considered reportable. Our results demonstrate the value of genome sequencing for routine clinical diagnosis but also highlight many outstanding challenges." https://pubmed.ncbi.nlm.nih.gov/25985138/

**20.Clark MM, Stark Z, Farnaes L, et al. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. NPJ Genom Med. 2018 Jul 9;3:16. PMID: 30002876**

- **Abstract:** "Genetic diseases are leading causes of childhood mortality. Whole-genome sequencing (WGS) and whole-exome sequencing (WES) are relatively new methods for diagnosing genetic diseases, whereas chromosomal microarray (CMA) is well established. Here we compared the diagnostic utility (rate of causative, pathogenic, or likely pathogenic genotypes in known disease genes) and clinical utility (proportion in whom medical or surgical management was changed by diagnosis) of WGS, WES, and CMA in children with suspected genetic diseases by systematic review of the literature

(January 2011-August 2017) and meta-analysis, following MOOSE/PRISMA guidelines. In 37 studies, comprising 20,068 children, diagnostic utility of WGS (0.41, 95% CI 0.34-0.48, I2 = 44%) and WES (0.36, 95% CI 0.33-0.40, I2 = 83%) were qualitatively greater than CMA (0.10, 95% CI 0.08-0.12, I2 = 81%). Among studies published in 2017, the diagnostic utility of WGS was significantly greater than CMA (P < 0.0001, I2 = 13% and I2 = 40%, respectively). Among studies featuring within-cohort comparisons, the diagnostic utility of WES was significantly greater than CMA (P < 0.001, I2 = 36%). The diagnostic utility of WGS and WES were not significantly different. In studies featuring within-cohort comparisons of WGS/WES, the likelihood of diagnosis was significantly greater for trios than singletons (odds ratio 2.04, 95% CI 1.62-2.56, I2 = 12%; P < 0.0001). Diagnostic utility of WGS/WES with hospital-based interpretation (0.42, 95% CI 0.38-0.45, I2 = 48%) was qualitatively higher than that of reference laboratories (0.29, 95% CI 0.27-0.31, I2 = 49%); this difference was significant among studies published in 2017 (P < .0001, I2 = 22% and I2 = 26%, respectively). The clinical utility of WGS (0.27, 95% CI 0.17-0.40, I2 = 54%) and WES (0.17, 95% CI 0.12-0.24, I2 = 76%) were higher than CMA (0.06, 95% CI 0.05-0.07, I2 = 42%); this difference was significant for WGS vs CMA (P < 0.0001). In conclusion, in children with suspected genetic diseases, the diagnostic and clinical utility of WGS/WES were greater than CMA. Subgroups with higher WGS/WES diagnostic utility were trios and those receiving hospital-based interpretation. WGS/WES should be considered a first-line genomic test for children with suspected genetic diseases." https://pubmed.ncbi.nlm.nih.gov/30002876/

**21.Shashi V, McConkie-Rosell A, Rosell B, et al The utility of the traditional medical genetics diagnostic evaluation in the context of next-generation sequencing for undiagnosed genetic disorders. Genet Med. 2014 Feb; 16: 176–182. PMID: 23928913**

- **Abstract:** "Purpose: The purpose of this study was to assess the diagnostic yield of the traditional, comprehensive clinical evaluation and targeted genetic testing, within a general genetics clinic. These data are critically needed to develop clinically and economically grounded diagnostic algorithms that consider presenting phenotype, traditional genetics testing, and the emerging role of next-generation sequencing (whole-exome/genome sequencing).
- **Methods**: We retrospectively analyzed a cohort of 500 unselected consecutive patients who received traditional genetic diagnostic evaluations at a tertiary medical center. We calculated the diagnosis rate, number of visits to diagnosis, genetic tests, and the cost of testing.
- **Results**: Thirty-nine patients were determined to not have a genetic disorder; 212 of the remaining 461 (46%) received a genetic diagnosis, and 72% of these were diagnosed on the first visit. The cost per subsequent successful genetic diagnosis was estimated at $25,000.
- **Conclusion**: Almost half of the patients were diagnosed using the traditional approach, most at the initial visit. For those remaining undiagnosed, next-generation sequencing may be clinically and economically beneficial. Estimating a 50% success rate for next-generation sequencing in undiagnosed genetic disorders, its application after the first clinical visit could result in a higher rate of genetic diagnosis at a considerable cost savings per successful diagnosis." https://pubmed.ncbi.nlm.nih.gov/23928913/

**22.Xue Y, Ankala A, Wilcox W, Hegde M. Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: single-gene, gene panel, or exome/genome sequencing. Genet Med. 2015 Jun;17(6):444-51. PMID: 25232854**

- **Abstract:** "Next-generation sequencing is changing the paradigm of clinical genetic testing. Today there are numerous molecular tests available, including single-gene tests, gene panels, and exome sequencing or genome sequencing. As a result, ordering physicians face the conundrum of selecting the best diagnostic tool for their patients with genetic conditions. Single-gene testing is often most appropriate for conditions with distinctive clinical features and minimal locus heterogeneity. Next-generation sequencing-based gene panel testing, which can be complemented with array comparative genomic hybridization and other ancillary methods, provides a comprehensive and feasible approach for heterogeneous disorders. Exome sequencing and genome sequencing have the advantage of being unbiased regarding what set of genes is analyzed, enabling parallel interrogation of most of the genes in the human genome. However, current limitations of next-generation sequencing technology and our variant interpretation capabilities caution us against offering exome sequencing or genome sequencing as either stand-alone or first-choice diagnostic approaches. A growing interest in personalized medicine calls for the application of genome sequencing in clinical diagnostics, but major challenges must be addressed before its full potential can be realized. Here, we propose a testing algorithm to help clinicians opt for the most appropriate molecular diagnostic tool for each scenario."
  https://pubmed.ncbi.nlm.nih.gov/25232854/

**23.ACMG Board of Directors. Points to consider in the clinical application of whole-genome sequencing. Genet Med 2012; 14:759–761. PMID 22863877**

- "Major advances in DNA sequencing technology have made it possible to do large-scale sequencing, up to and including whole-genome sequencing (WGS), in an effort to identify a gene mutation that may provide a diagnosis for a patient with an abnormal phenotype. This strategy offers potential advantages over classic approaches in which genes are analyzed individually, often over a long period of time and at substantial expense. As a result, there is considerable interest in offering genomic sequencing–based tests on a clinical basis. This document outlines points to consider in the clinical application of genomic sequencing to the detection of germ-line mutations. It is expected that this document will require revision as this rapidly changing field evolves."
  https://www.gimjournal.org/action/showPdf?pii=S1098-3600%2821%2902298-X

**24.Manickam K, McClain MR, Demmer LA, et al. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021 Nov;23(11):2029-2037. PMID: 34211152**

- **Abstract:** "Purpose: To develop an evidence-based clinical practice guideline for the use of exome and genome sequencing (ES/GS) in the care of pediatric patients with one or

more congenital anomalies (CA) with onset prior to age 1 year or developmental delay (DD) or intellectual disability (ID) with onset prior to age 18 years.

- **Methods**: The Pediatric Exome/Genome Sequencing Evidence-Based Guideline Work Group (n = 10) used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) evidence to decision (EtD) framework based on the recent American College of Medical Genetics and Genomics (ACMG) systematic review, and an Ontario Health Technology Assessment to develop and present evidence summaries and health-care recommendations. The document underwent extensive internal and external peer review, and public comment, before approval by the ACMG Board of Directors.
- **Results**: The literature supports the clinical utility and desirable effects of ES/GS on active and long-term clinical management of patients with CA/DD/ID, and on family-focused and reproductive outcomes with relatively few harms. Compared with standard genetic testing, ES/GS has a higher diagnostic yield and may be more cost-effective when ordered early in the diagnostic evaluation.
- **Conclusion**: We strongly recommend that ES/GS be considered as a first- or second-tier test for patients with CA/DD/ID." https://pubmed.ncbi.nlm.nih.gov/34211152/

**25.ACMG Board of Directors. Points to consider for informed consent for genome/exome sequencing. Genet Med Sept 2013;15(9):748–749. PMID 23970068**

- **Summary:** A document that provides recommendations for obtaining consent before genome/exome sequencing. https://www.gimjournal.org/action/showPdf?pii=S1098-3600%2821%2902732-5

**26.Rehder C, Bean LJH, Bick D, et al. Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021 Aug;23(8):1399-1415. PMID: 33927380**

- **Abstract:** "Next-generation sequencing (NGS) technologies are now established in clinical laboratories as a primary testing modality in genomic medicine. These technologies have reduced the cost of large-scale sequencing by several orders of magnitude. It is now cost-effective to analyze an individual with disease-targeted gene panels, exome sequencing, or genome sequencing to assist in the diagnosis of a wide array of clinical scenarios. While clinical validation and use of NGS in many settings is established, there are continuing challenges as technologies and the associated informatics evolve. To assist clinical laboratories with the validation of NGS methods and platforms, the ongoing monitoring of NGS testing to ensure quality results, and the interpretation and reporting of variants found using these technologies, the American College of Medical Genetics and Genomics (ACMG) has developed the following technical standards." https://pubmed.ncbi.nlm.nih.gov/33927380/

**27.Miller DT, Lee K, Gordon AS, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of**

**the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021 Aug;23(8):1391-1398. PMID: 34012069**.

- **Summary**: An article that provides policy updates and clarification on secondary findings on clinical exome and genome sequencing. https://www.gimjournal.org/action/showPdf?pii=S1098-3600%2821%2905075-9

**28.Miller DT, Lee K, Chung WK, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021 Aug;23(8):1381-1390. Erratum in: Genet Med. 2021 Aug 3. PMID: 34012068**

- **Introduction:** "The American College of Medical Genetics and Genomics (ACMG)previously published guidance for reporting secondary findings in the context of clinical exome and genome sequencing (ES/GS) in2013 and 2017.1,2 These recommendations were developed by the ACMG Secondary Findings Maintenance Working Group (SFWG), which was convened by the ACMG Board of Directors (BOD) to evaluate the need for a minimum list of genes that should be evaluated in individuals undergoing clinical ES/GS based on the medical actionability of the associated condition. In the past, policy recommendations concerning what types of variants to return along with lists of which genes to analyze were included. Given the increase in uptake of clinical ES/GS, the ACMG SFWG and BOD have agreed the list of recommended genes should now be updated annually. Policy updates surrounding the purpose, scope, and process for maintaining the ACMG Secondary Findings List are being published separately,3 and will be updated separately, as needed. It is important to reiterate here that use of the SF results should not be a replacement for indication-based diagnostic clinical genetic testing." https://www.gimjournal.org/action/showPdf?pii=S1098-3600%2821%2905076-0

**29.Deignan JL, Chung WK, Kearney HM, et al. Points to consider in the reevaluation and reanalysis of genomic test results: a statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2019 Jun;21(6):1267-1270. PMID: 31015575**

- **Abstract:** "Clinicians are encouraged to document the reasons for the use of a particular procedure or test, whether or not it is in conformance with this statement. Clinicians also are advised to take notice of the date this statement was adopted, and to consider other medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures. Where individual authors are listed, the views expressed may not reflect those of authors' employers or affiliated institutions." https://pubmed.ncbi.nlm.nih.gov/37347242/

**30.Schwarze K, Buchanan J, Taylor J, et al. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genet Med. 2018 Oct;20(10):1122-1130. PMID: 29446766**

- **Abstract:** "Purpose: We conducted a systematic literature review to summarize the current health economic evidence for whole-exome sequencing (WES) and whole-genome sequencing (WGS).

- **Methods**: Relevant studies were identified in the EMBASE, MEDLINE, Cochrane Library, EconLit and University of York Centre for Reviews and Dissemination databases from January 2005 to July 2016. Publications were included in the review if they were economic evaluations, cost studies, or outcome studies.
- **Results**: Thirty-six studies met our inclusion criteria. These publications investigated the use of WES and WGS in a variety of genetic conditions in clinical practice, the most common being neurological or neurodevelopmental disorders. Study sample size varied from a single child to 2,000 patients. Cost estimates for a single test ranged from $555 to $5,169 for WES and from $1,906 to $24,810 for WGS. Few cost analyses presented data transparently and many publications did not state which components were included in cost estimates.
- **Conclusion**: The current health economic evidence base to support the more widespread use of WES and WGS in clinical practice is very limited. Studies that carefully evaluate the costs, effectiveness, and cost-effectiveness of these tests are urgently needed to support their translation into clinical practice." https://pubmed.ncbi.nlm.nih.gov/29446766/

**31.Splinter K, Adams DR, Bacino CA, et al. Effect of Genetic Diagnosis on Patients with Previously Undiagnosed Disease. N Engl J Med. 2018 Nov 29;379(22):2131-2139. PMID: 30304647**

- **Abstract:** "Background: Many patients remain without a diagnosis despite extensive medical evaluation. The Undiagnosed Diseases Network (UDN) was established to apply a multidisciplinary model in the evaluation of the most challenging cases and to identify the biologic characteristics of newly discovered diseases. The UDN, which is funded by the National Institutes of Health, was formed in 2014 as a network of seven clinical sites, two sequencing cores, and a coordinating center. Later, a central biorepository, a metabolomics core, and a model organisms screening center were added.
- **Methods**: We evaluated patients who were referred to the UDN over a period of 20 months. The patients were required to have an undiagnosed condition despite thorough evaluation by a health care provider. We determined the rate of diagnosis among patients who subsequently had a complete evaluation, and we observed the effect of diagnosis on medical care.
- **Results**: A total of 1519 patients (53% female) were referred to the UDN, of whom 601 (40%) were accepted for evaluation. Of the accepted patients, 192 (32%) had previously undergone exome sequencing. Symptoms were neurologic in 40% of the applicants, musculoskeletal in 10%, immunologic in 7%, gastrointestinal in 7%, and rheumatologic in 6%. Of the 382 patients who had a complete evaluation, 132 received a diagnosis, yielding a rate of diagnosis of 35%. A total of 15 diagnoses (11%) were made by clinical review alone, and 98 (74%) were made by exome or genome sequencing. Of the diagnoses, 21% led to recommendations regarding changes in therapy, 37% led to changes in diagnostic testing, and 36% led to variant-specific genetic counseling. We defined 31 new syndromes.

- **Conclusions**: The UDN established a diagnosis in 132 of the 382 patients who had a complete evaluation, yielding a rate of diagnosis of 35%." https://pubmed.ncbi.nlm.nih.gov/30304647/

**32.Scocchia A, Wigby KM, Masser-Frye D, et al. Clinical whole genome sequencing as a first-tier test at a resource-limited dysmorphology clinic in Mexico. NPJ Genom Med. 2019 Feb 14;4:5. PMID: 30792901**

- **Abstract:** "Patients with rare, undiagnosed, or genetic disease (RUGD) often undergo years of serial testing, commonly referred to as the "diagnostic odyssey". Patients in resource-limited areas face even greater challenges-a definitive diagnosis may never be reached due to difficulties in gaining access to clinicians, appropriate specialists, and diagnostic testing. Here, we report on a collaboration of the Illumina iHope Program with the Foundation for the Children of the Californias and Hospital Infantil de Las Californias, to enable deployment of clinical whole genome sequencing (cWGS) as first-tier test in a resource-limited dysmorphology clinic in northern Mexico. A total of 60 probands who were followed for a suspected genetic diagnosis and clinically unresolved after expert examination were tested with cWGS, and the ordering clinicians completed a semi-structured survey to investigate change in clinical management resulting from cWGS findings. Clinically significant genomic findings were identified in 68.3% (n = 41) of probands. No recurrent molecular diagnoses were observed. Copy number variants or gross chromosomal abnormalities accounted for 48.8% (n = 20) of the diagnosed cases, including a mosaic trisomy and suspected derivative chromosomes. A qualitative assessment of clinical management revealed 48.8% (n = 20) of those diagnosed had a change in clinical course based on their cWGS results, despite resource limitations. These data suggest that a cWGS first-tier testing approach can benefit patients with suspected genetic disorders." https://pubmed.ncbi.nlm.nih.gov/30792901/

**33.Kingsmore, S.F., Nofsinger, R. & Ellsworth, K. Rapid genomic sequencing for genetic disease diagnosis and therapy in intensive care units: a review. npj Genom. Med. 9, 17 (2024). https://doi.org/10.1038/s41525-024-00404-0**

- **Abstract:** "Single locus (Mendelian) diseases are a leading cause of childhood hospitalization, intensive care unit (ICU) admission, mortality, and healthcare cost. Rapid genome sequencing (RGS), ultra-rapid genome sequencing (URGS), and rapid exome sequencing (RES) are diagnostic tests for genetic diseases for ICU patients. In 44 studies of children in ICUs with diseases of unknown etiology, 37% received a genetic diagnosis, 26% had consequent changes in management, and net healthcare costs were reduced by $14,265 per child tested by URGS, RGS, or RES. URGS outperformed RGS and RES with faster time to diagnosis, and higher rate of diagnosis and clinical utility. Diagnostic and clinical outcomes will improve as methods evolve, costs decrease, and testing is implemented within precision medicine delivery systems attuned to ICU needs. URGS, RGS, and RES are currently performed in <5% of the ~200,000 children likely to benefit annually due to lack of payor coverage, inadequate reimbursement, hospital policies, hospitalist unfamiliarity, under-recognition of possible genetic diseases, and current formatting as tests rather than as a rapid precision medicine delivery system. The gap between actual and optimal outcomes in children in ICUs is currently increasing since expanded use of URGS, RGS, and RES lags growth in those likely to

benefit through new therapies. There is sufficient evidence to conclude that URGS, RGS, or RES should be considered in all children with diseases of uncertain etiology at ICU admission. Minimally, diagnostic URGS, RGS, or RES should be ordered early during admissions of critically ill infants and children with suspected genetic diseases." https://pubmed.ncbi.nlm.nih.gov/38413639/

**34.Wojcik, M.H., et al. Genome Sequencing for Diagnosing Rare Diseases. The New England Journal of Medicine. https://www.nejm.org/doi/full/10.1056/NEJMoa2314761**

- **Abstract:** "Background: Genetic variants that cause rare disorders may remain elusive even after expansive testing, such as exome sequencing. The diagnostic yield of genome sequencing, particularly after a negative evaluation, remains poorly defined.
- **Methods**: We sequenced and analyzed the genomes of families with diverse phenotypes who were suspected to have a rare monogenic disease and for whom genetic testing had not revealed a diagnosis, as well as the genomes of a replication cohort at an independent clinical center.
- **Results**: We sequenced the genomes of 822 families (744 in the initial cohort and 78 in the replication cohort) and made a molecular diagnosis in 218 of 744 families (29.3%). Of the 218 families, 61 (28.0%) - 8.2% of families in the initial cohort - had variants that required genome sequencing for identification, including coding variants, intronic variants, small structural variants, copy-neutral inversions, complex rearrangements, and tandem repeat expansions. Most families in which a molecular diagnosis was made after previous nondiagnostic exome sequencing (63.5%) had variants that could be detected by reanalysis of the exome-sequence data (53.4%) or by additional analytic methods, such as copy-number variant calling, to exome-sequence data (10.8%). We obtained similar results in the replication cohort: in 33% of the families in which a molecular diagnosis was made, or 8% of the cohort, genome sequencing was required, which showed the applicability of these findings to both research and clinical environments.
- **Conclusions**: The diagnostic yield of genome sequencing in a large, diverse research cohort and in a small clinical cohort of persons who had previously undergone genetic testing was approximately 8% and included several types of pathogenic variation that had not previously been detected by means of exome sequencing or other techniques." https://pubmed.ncbi.nlm.nih.gov/38838312/

**35.Wojcik, M.H., et al. Beyond the exome: What's next in diagnostic testing for Mendelian conditions. The American Journal of Human Genetics *110,* 1229-1248, August 3, 2023. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9882576/**

- **Abstract:** "Despite advances in clinical genetic testing, including the introduction of exome sequencing (ES), more than 50% of individuals with a suspected Mendelian condition lack a precise molecular diagnosis. Clinical evaluation is increasingly undertaken by specialists outside of clinical genetics, often occurring in a tiered fashion and typically ending after ES. The current diagnostic rate reflects multiple factors, including technical limitations, incomplete understanding of variant pathogenicity, missing genotype-phenotype associations, complex gene-environment interactions, and reporting differences between clinical labs. Maintaining a clear understanding of the

rapidly evolving landscape of diagnostic tests beyond ES, and their limitations, presents a challenge for non-genetics professionals. Newer tests, such as short-read genome or RNA sequencing, can be challenging to order and emerging technologies, such as optical genome mapping and long-read DNA or RNA sequencing, are not available clinically. Furthermore, there is no clear guidance on the next best steps after inconclusive evaluation. Here, we review why a clinical genetic evaluation may be negative, discuss questions to be asked in this setting, and provide a framework for further investigation, including the advantages and disadvantages of new approaches that are nascent in the clinical sphere. We present a guide for the next best steps after inconclusive molecular testing based upon phenotype and prior evaluation, including when to consider referral to a consortium such as GREGoR, which is focused on elucidating the underlying cause of rare unsolved genetic disorders."
https://pubmed.ncbi.nlm.nih.gov/36713248/

**36.Lowther et al. Systematic evaluation of genome sequencing for the diagnostic assessment of autism spectrum disorder and fetal structural anomalies.**

- **Abstract:** "Short-read genome sequencing (GS) holds the promise of becoming the primary diagnostic approach for the assessment of autism spectrum disorder (ASD) and fetal structural anomalies (FSAs). However, few studies have comprehensively evaluated its performance against current standard-of-care diagnostic tests: karyotype, chromosomal microarray (CMA), and exome sequencing (ES). To assess the clinical utility of GS, we compared its diagnostic yield against these three tests in 1,612 quartet families including an individual with ASD and in 295 prenatal families. Our GS analytic framework identified a diagnostic variant in 7.8% of ASD probands, almost 2-fold more than CMA (4.3%) and 3-fold more than ES (2.7%). However, when we systematically captured copy-number variants (CNVs) from the exome data, the diagnostic yield of ES (7.4%) was brought much closer to, but did not surpass, GS. Similarly, we estimated that GS could achieve an overall diagnostic yield of 46.1% in unselected FSAs, representing a 17.2% increased yield over karyotype, 14.1% over CMA, and 4.1% over ES with CNV calling or 36.1% increase without CNV discovery. Overall, GS provided an added diagnostic yield of 0.4% and 0.8% beyond the combination of all three standard-of-care tests in ASD and FSAs, respectively. This corresponded to nine GS unique diagnostic variants, including sequence variants in exons not captured by ES, structural variants (SVs) inaccessible to existing standard-of-care tests, and SVs where the resolution of GS changed variant classification. Overall, this large-scale evaluation demonstrated that GS significantly outperforms each individual standard-of-care test while also outperforming the combination of all three tests, thus warranting consideration as the first-tier diagnostic approach for the assessment of ASD and FSAs."
https://pubmed.ncbi.nlm.nih.gov/37595579/

**37.Jobanputra, V., et al. Advancing access to genome sequencing for rare genetic disorders: recent progress and call to action. Npj Genom. Med. 9, 23 (2024).**

- **Introduction:** "Epidemiologic studies estimate that 2–6% of the global population is affected by a rare disease, up to 80% of which are genetic in origin. Diagnostic delays can result in significant burdens including missed opportunities for intervention, unnecessary procedures and treatments, and an emotional toll on families and their care

providers. Genome sequencing (GS) provides a comprehensive profile of genetic variants associated with disease, including assessment of single nucleotide variants (SNV), indels, copy-number and structural variants, repeat expansions, and mitochondrial genome variation. The diagnostic potential of GS is underscored by the increasing evidence that it can end the so-called diagnostic odyssey for up to ~20-60% of neonates and ~17-40% of pediatric patients with a suspected genetic disease. GS testing often leads to measurable changes in management, with studies suggesting that up to 77% of patients receive a change in care as a result of receiving diagnostic genome findings. Health economic studies examining the incremental net benefit of GS in comparison to other genetic tests indicate that first-line GS can be a cost-effective strategy in patients with suspected rare diseases. These and other results have led the Medical Genome Initiative to argue that GS should be applied as a first-line test for patients with a suspected rare genetic disease, and have supported the inclusion of GS in clinical practice guidelines published by the American College of Medical Genetics and Genomics (ACMG) in 202113 and the European Society for Human Genetics (ESHG) in 202214. Until recently, however, there has been limited government and payer support of GS testing." https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10973466/