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## Whole Exome and Whole Genome Sequencing (for Louisiana Only)

**Policy Number:** CS150LA.# I  
**Effective Date:** ~~October 1, 2022~~ TBD

[Instructions for Use](#)

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### Application

This Medical Policy only applies to the state of Louisiana.

### Coverage Rationale

#### State-Specific Criteria

The coverage criteria for genetic counseling contained in this policy represents Louisiana Medicaid Managed Care Organization Manual (LA MCO) coverage policy and is set forth below in accordance with State requirements.

#### Genetic Counseling

Genetic counseling before and after all genetic testing is required. Counseling must consist of at least all of the following and be documented in the medical record:

- Obtaining a structured family genetic history
- Genetic risk assessment; and
- Counseling of the enrollee and family about diagnosis, prognosis, and treatment (LA MCO Genetic Counseling and Testing, page 112)

~~Genetic counseling is strongly recommended prior to these tests in order to inform persons being tested about the advantages and limitations of the test as applied to a unique person.~~

## **Additional Non State-Specific Criteria**

### **Whole Exome Sequencing (WES)**

Whole Exome Sequencing (WES) is proven and ~~m~~ Medically necessary~~Necessary~~~~medically necessary~~ for the following:

- Diagnosing ~~diagnosing~~ or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes and **all** of the following criteria are met:
  - Clinical presentation is nonspecific and does not fit a well-defined syndrome for which a specific or targeted gene test is available. If a specific genetic syndrome is suspected, a single gene or targeted gene panel should be performed prior to determining if WES is necessary; and
  - WES is ordered by a ~~board-certified~~ medical geneticist, neonatologist, neurologist, or developmental ~~and behavioral~~ pediatrician; and
  - One of the following:
    - ~~Clinical~~ ~~The clinical presentation or clinical and/or family history strongly suggests~~ ~~suggest~~ a genetic cause ~~for which a specific clinical diagnosis cannot be made with any clinically available targeted genetic tests~~ and one or more of the following features are present: ~~or~~
      - Multiple congenital anomalies (must affect different organ systems)
      - Moderate, severe, or profound Intellectual Disability diagnosed by 18 years ~~There is a clinical diagnosis of age~~
      - Global Developmental Delay
      - Epileptic encephalopathy with onset before three years of age; or
      - Clinical history strongly suggests a ~~a~~ genetic cause and two or more of the following features are present:
        - Congenital anomaly
        - Significant hearing or visual impairment diagnosed by 18 years of age
        - Laboratory abnormalities suggestive of an inborn error of metabolism (IEM)
        - Autism spectrum disorder
        - Neuropsychiatric condition (e.g., bipolar disorder, schizophrenia, obsessive-compulsive disorder)
        - Hypotonia or hypertonia in infancy
        - Dystonia, ataxia, hemiplegia, neuromuscular disorder, movement disorder, or other neurologic abnormality
        - Unexplained developmental regression, unrelated to autism or epilepsy
        - Growth abnormality (e.g., failure to thrive, short stature, microcephaly, macrocephaly, or overgrowth)
        - Persistent and severe immunologic or hematologic disorder
        - Dysmorphic features
        - Consanguinity
        - Other first- or second-degree family member(s) with similar clinical features
    - ~~where there is significant genetic heterogeneity and WES is a more practical approach to identifying the underlying genetic cause than are individual tests of multiple genes; or~~
    - ~~There is likely a genetic disorder and multiple targeted gene tests that have failed to identify the underlying cause~~

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- Comparator (e.g., parents or siblings) WES ~~is proven and medically necessary~~ for evaluating a genetic disorder when the above criteria have been met and WES is performed concurrently or has been previously performed on the individual.
- Reanalysis of WES after at least 18 months when above criteria for initial WES has been met and one of the following occurs:
  - Individual experiences additional symptoms after initial WES that cannot be explained by the results of the initial WES; or
  - New data or new family history emerges which suggest a link between the individual's symptoms and specific genes

**Due to insufficient evidence of efficacy,**

WES is unproven and not ~~Medically Necessary~~ for all other indications, including but not limited to the following:

- ~~Screening and evaluating disorders in individuals when the above criteria are not met~~
- ~~Prenatal genetic diagnosis or screening~~
- ~~Evaluation of fetal demise~~
- ~~Preimplantation Genetic Testing (PGT) in embryos~~
- Molecular profiling of tumors for the diagnosis, prognosis or management of cancer
- ~~Preimplantation Genetic Testing (PGT) in embryos~~

~~Further studies are needed to evaluate the clinical utility of whole exome sequencing for other indications.~~

- Prenatal genetic diagnosis or screening
- Screening and evaluating disorders in individuals when the above criteria are not met

## **Whole Genome Sequencing (WGS)**

Whole Genome Sequencing (WGS) is Medically Necessary~~unproven and not medically necessary~~ for the following:

- Diagnosing or ~~screening and~~ evaluating a genetic~~any~~ disorder when the results are expected to directly influence medical management and clinical outcomes and all of the following criteria are met:-
  - Neither chromosome microarray analysis (CMA) nor WES have been performed; and
  - Clinical presentation is nonspecific and does not fit a well-defined syndrome for which a specific or targeted gene test is available. If a specific genetic syndrome is suspected, a single gene or targeted gene panel should be performed prior to determining if WGS is necessary; and
  - WGS is ordered by a medical geneticist, neonatologist, neurologist, or developmental pediatrician; and
  - One of the following:
    - Clinical history strongly suggests a genetic cause and one or more of the following features are present:
      - Multiple congenital anomalies (must affect different organ systems)
      - Moderate, severe, or profound Intellectual Disability diagnosed by 18 years of age
      - Global Developmental Delay
      - Epileptic encephalopathy with onset before three years of age; or
    - Clinical history strongly suggests a genetic cause and two or more of the following features are present:

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- Congenital anomaly
  - Significant hearing or visual impairment diagnosed by 18 years of age
  - Laboratory abnormalities suggestive of an IEM
  - Autism spectrum disorder
  - Neuropsychiatric condition (e.g., bipolar disorder, schizophrenia, obsessive-compulsive disorder)
  - Hypotonia or hypertonia in infancy
  - Dystonia, ataxia, hemiplegia, neuromuscular disorder, movement disorder, or other neurologic abnormality
  - Unexplained developmental regression, unrelated to autism or epilepsy
  - Growth abnormality (e.g., failure to thrive, short stature, microcephaly, macrocephaly, or overgrowth)
  - Persistent and severe immunologic or hematologic disorder
  - Dysmorphic features
  - Consanguinity
  - Other first- or second-degree family member(s) with similar clinical features
- Comparator (e.g., parents or siblings) WGS for evaluating a genetic disorder when the above criteria have been met and WGS is performed concurrently or has been previously performed on the individual

~~Whole Genome Sequencing (WGS) is not Medically Necessary for evaluating any genetic disorder in any other clinical situation due to the availability of clinically equivalent diagnostic tests.~~

~~\*This policy applies to genetic testing in an outpatient setting or upon discharge from an inpatient setting. Note: The evaluation of cancer is addressed in the Medical Policy titled Molecular Oncology Testing for Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only). Additionally, this policy for Whole Exome and Whole Genome Sequencing is limited to genetic testing in an outpatient setting or upon discharge from an inpatient setting. Although WGS has the potential to identify causal variants for a wide variety of conditions that may be missed with other technologies, as well as to identify predictive biomarkers, the information derived from WGS has not yet been translated into improved outcomes and changed medical management. Further studies are needed to establish the clinical utility of WGS.~~

## Definitions

**Comparator:** A DNA sequence that is used to compare to the individual's ~~patient's~~ DNA sequence. This may be a parent or sibling of the individual~~patient~~, or non-cancerous tissue that is being compared to the individual's ~~patient's~~ tumor tissue (Thun et al., 2017).

Consanguinity: Procreation with second-cousins or closer (Bennett et al., 2021).

Global Developmental Delay: A significant delay in 2 or more developmental domains, including gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living with onset prior to 5 years of age (Shevell et al., 2003).

Intellectual Disability: a condition diagnosed before age 18 that includes below-average intellectual function and a lack of skills necessary for daily living (MedlinePlus, 2020a).

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**Medically Necessary:** Health care services that are all of the following as determined by us or our designee:

- In accordance with Generally Accepted Standards of Medical Practice.
- Clinically appropriate, in terms of type, frequency, extent, service site and duration, and considered effective for your Sickness, Injury, Mental Illness, substance-related and addictive disorders, disease or its symptoms.
- Not mainly for your convenience or that of your doctor or other health care provider.
- Not more costly than an alternative drug, service(s), service site or supply that is at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of your Sickness, Injury, disease or symptoms.

Generally Accepted Standards of Medical Practice are standards that are based on credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community, relying primarily on controlled clinical trials, or, if not available, observational studies from more than one institution that suggest a causal relationship between the service or treatment and health outcomes.

If no credible scientific evidence is available, then standards that are based on Physician specialty society recommendations or professional standards of care may be considered. We have the right to consult expert opinion in determining whether health care services are Medically Necessary. The decision to apply Physician specialty society recommendations, the choice of expert and the determination of when to use any such expert opinion, shall be determined by us (UnitedHealthcare Insurance Company Generic Certificate of Coverage 2018).

**Next Generation Sequencing (NGS):** New sequencing techniques that can quickly analyze multiple sections of DNA at the same time. Older forms of sequencing could only analyze one section of DNA at once.

**Preimplantation Genetic Testing (PGT):** A test performed to analyze the DNA from oocytes or embryos for human leukocyte antigen (HLA)-typing or for determining genetic abnormalities. These include:

- PGT-A: For aneuploidy screening (formerly PGS)
- PGT-M: For monogenic/single gene defects (formerly single-gene PGD)
- PGT-SR: For chromosomal structural rearrangements (formerly chromosomal PGD) (Zegers-Hochschild et al., 2017)

**Variant of Unknown Significance (VUS):** A variation in a genetic sequence that has an unknown association with disease. It may also be called an unclassified variant.

**Whole Exome Sequencing (WES):** About 1% of a person's DNA makes protein. These protein making sections are called exons. All the exons together are called the exome. WES is a DNA analysis technique that looks at all of the exons in a person at one time, rather than gene by gene (~~U.S. National Library of Medicine, What are whole exome sequencing and whole genome sequencing? 2018~~). MedlinePlus, 2020b).

**Whole Genome Sequencing (WGS):** WGS determines the sequence of all of the DNA in a person, which includes the protein making (coding) as well as non-coding DNA elements (~~U.S. National Library of Medicine, What are whole exome sequencing and whole genome sequencing? 2018~~). MedlinePlus, 2020b).

## Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by federal, state or contractual requirements and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and ~~Coverage Determination~~ Guidelines may apply.

| CPT Code      | Description  |
|---------------|--|
| <u>*0036U</u> | Exome (i.e., somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses   |
| <u>*0094U</u> | Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis   |
| <u>*0212U</u> | <u>Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband</u>  |
| <u>*0213U</u> | <u>Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator genome (e.g., parent, sibling)</u>   |
| <u>*0214U</u> | <u>Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband</u>   |
| <u>*0215U</u> | <u>Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator exome (e.g., parent, sibling)</u>   |
| <u>*0265U</u> | Rare constitutional and other heritable disorders, whole genome and mitochondrial DNA sequence analysis, blood, frozen and formalin-fixed paraffin-embedded (FFPE) tissue, saliva, buccal swabs or cell lines, identification of single nucleotide and copy number variants  |
| <u>*0335U</u> | Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, fetal sample, identification and categorization of genetic variants |

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| CPT Code | Description  |
|----------|--|
| *0336U   | Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (e.g., parent) |
| *81415   | Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis  |
| *81416   | Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (e.g., parents, siblings) (List separately in addition to code for primary procedure)   |
| *81417   | Exome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (e.g., updated knowledge or unrelated condition/syndrome)  |
| *81425   | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis   |
| *81426   | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (e.g., parents, siblings) (List separately in addition to code for primary procedure)   |
| *81427   | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (e.g., updated knowledge or unrelated condition/syndrome)  |

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**Codes labeled with an asterisk (\*) are not on the Louisiana Medicaid Fee Schedule and therefore may not be covered by the state of Louisiana Medicaid Program.**

## Description of Services

~~Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES)~~ Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES) are increasingly clinically available due to significant advances in DNA sequencing technology over the last several years (Taber et al., 2014). ~~This testing approach, when applied to appropriate individuals and ordered and interpreted by medical specialists, can save both time and resources for individuals and their families [Beale et al., 2015; Frank et al., 2013; Canadian Agency for Drugs and Technologies in Health, 2014; American College of Medical Genetics and Genomics (ACMG), 2013].~~

WES refers to the sequence determination of the exome. The exome is the portion of an individual's genome that encodes protein (also known as exons).

~~Most known disease-Approximately 1% of the genome is comprised of exons, which is about 30 million base pairs (Bertier, et al., 2016) and between 20,000-25,000 genes (U.S. National Library of Medicine, What is a gene? 2018). Most known disease-causing variants are found in the exons, and by sequencing them all simultaneously, a more efficient analysis can be completed than by sequencing each individual gene alone (Bertier et al., 2016). There is much that is unknown, however, and to date only 3,911 genes that are known to harbor one or more disease causing mutations have been identified (Online Mendelian Inheritance in Man, 2018).~~



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WES results in long lists of genetic variants, and the success of this technology is dependent on how consistently and accurately labs can identify disease causing mutations (Richards et al., 2015).

~~The Clinical Sequencing Exploratory Research Consortium (CSER) studied variant assessment between nine labs performing exome analysis and applying the ACMG and AMP sequence variant interpretation guidelines, and found that intra lab concordance was 79%, but inter lab concordance was only 34%. After consensus efforts, 70% concordance was achieved between labs, reflecting the continued subjectivity. Five percent of the discordant interpretations would impact clinical care (Green et al., 2016).~~

~~In addition, because all genes are being analyzed simultaneously, an unexpected or incidental finding may be identified in the analysis that was outside of the clinical indication for the test (Richards et al., 2015). Novel variants may be discovered for the first time in the context of clinical care, laboratories that perform WES are in the unique position of requiring detailed clinical information to interpret results, and that may occasionally include testing of biological relatives (Richards et al., 2015). WES may result in false positives due to the difficulty in reading CG rich regions, and poor coverage depth (Meienberg et al., 2016). A comparison of standard next generation sequencing (NGS) techniques and WES demonstrated that > 98% of pathogenic variants are covered at depth adequate for detection (LaDuca et al., 2017).~~

~~For these reasons, it is critical that the ordering physician has specialty training and experience with these technologies and is prepared to work with the laboratory and interpret the results of such testing for their patient (Taber et al., 2014; Richards, et al., 2015; ACMG, 2012).~~

WGS determines the order of all the nucleotides in an individual's DNA and can determine variations in any part of the genome (U.S. Library of Medicine MedlinePlus, 2020b ~~what, What are Whole Exome Sequencing~~ whole exome sequencing and Whole Genome Sequencing ~~whole genome sequencing?~~ 2018). As with WES, WGS results in long lists of unknown variants., and The the methodology and databases available to interpret WGS are the same as WES, and focuses primarily on the exons (Richards et al., 2015; Landrum et al., 20156).

~~The functional implications of variants outside the exons are relatively unknown (Klein and Faroud, 2017). To date, only a small number of research articles have addressed the clinical utility of WGS. Recently several small studies have addressed the analytical validity of WGS as compared to WES, and found that WGS may provide more uniform coverage than WES, may more accurately detect a small number of variants compared to WES, and be better at identifying copy number variants (Belkadi et al., 2015; Meienberg et al., 2016). However, the data complexity, processing time, and interpretation time is much greater for WGS than for other NGS approaches (Klein and Faroud, 2017).~~

## Clinical Evidence

### Whole Exome Sequencing (WES)

#### WES: Pediatric (Non-Cancer)

In a 2022 Clinical Utility Evaluation, Hayes found insufficient evidence for use of WES to assist with clinical decision-making and improve overall outcomes in adults with suspected neuromuscular disease or movement disorders. Hayes notes that only limited,



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very low -quality evidence is available at this time and larger prospective studies investigating impact on clinical management and outcomes are required.

Sánchez-Luquez et al. (2022) sought to estimate the rate of molecular diagnostic assessment of intellectual disability (ID) by WES, quantify the amount of de novo mutations (DNMs) that contribute to that rate and attempt to characterize the genes related to the mutations found through WES in their recent systematic review and meta-analysis. Studies published between 2010 and 2022 were searched and ultimately 37 articles with information on molecular diagnostic yield using WES for ID were included. The diagnostic rate for WES was found to be 42% (Confidence interval [CI]: 35-50%), and the estimate related to DNMs only was 11% (CI: 6-18%). The diagnostic yield was significantly greater when testing of both biological parents was done or multiple affected family members were tested. The rate specific to DNMs supports the utility of WES for unexplained ID. The authors assert that the use of WES for molecular diagnosis of ID is supported by the results of this review. Authors Ewans (2018) and Bowling (2017), previously cited in this policy, were included in the Sánchez-Luquez systematic review.

To compare the yield of genetic testing across both sequencing technologies and subtypes of neurodevelopmental disorders, Stefanski et al. (2021) performed a systematic review and meta-analysis of studies using next generation sequencing (NGS) for individuals with autism spectrum disorder (ASD), epilepsy and ID. After applying selection criteria, 103 studies (ASD N=14, ID N=21, epilepsy N=72) including results for 32,331 individuals were analyzed. In 36 study groups, exome sequencing (ES) was used and in 73 groups targeted gene panel sequencing was used. The diagnostic yield was 23.7% overall; for ASD, epilepsy and ID, yields were 17.1% 24% and 28.2%, respectively. Authors note that the highest diagnostic yield for those with epilepsy was found in individuals with ID and early onset seizures. Although the diagnostic yield for ES was higher than for panel sequencing, the difference was not statically significant (27.2% vs 22.6%, P = .071). Per these results, approximately 1/5<sup>th</sup> of individuals with NDD can receive a molecular diagnosis using NGS. Further discussed is a potential explanation for the lower diagnostic yield found in this review compared to previous studies. The researchers suggest that study composition may have played a role; this systematic review included three to four times the number of studies compared to other reviews done. In addition, only studies with a minimum of 20 participants were used, increasing statistical accuracy, and this review included panel based studies in addition to ES data. Limitations of this review include potential for underestimation of diagnostic yield related to the use of standard genetic tests prior to NGS in some studies. Also, not all of the studies used ACMG classification guidelines for variants and the studies consisted of a heterogeneous collection of methodologies for sample and data collection. Lastly, generalizability to a global population is limited, as no studies from Africa, India or Latin America were included. Additional randomized controlled studies focused on evidence for the types of genetic testing that will best serve the need of afflicted individuals are recommended. In spite of the limitations, this study is the largest meta-analysis investigating diagnostic yield for NGS to date and provides comprehensive data regarding the use of NGS for NDD to assist with management of individuals with these disorders.

A small but growing body of evidence suggests that some cases of cerebral palsy may be attributable to rare genomic variants including copy number variants (CNVs) and single nucleotide variants (SNVs). To further investigate the molecular diagnostic yield of exome sequencing in individuals with cerebral palsy, Moreno-De-Luca et al. (2021) conducted a retrospective cohort study. The study included 2 cohorts of 1526 participants total with cerebral palsy; 1345 were included in the cohort referred to as the clinical laboratory referral cohort, and 181 were included in the cohort called the health care-

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based cohort. The clinical laboratory referral cohort had a median age of 8.8 years, and the health care-based cohort had a median age of 41.9 years. In the clinical laboratory referral cohort (predominantly pediatric), molecular diagnostic yield of exome sequencing was 32.7% and in the health care-based cohort (predominantly adult), it was 10.5%. Pathogenic or likely pathogenic variants were identified in 229 genes; 86 genes were mutated in 2 or more ~~patients~~ participants and 10 genes with mutations were found independently in both cohorts. Noted limitations include the variation in capture reagents for sequencing, variability in clinical information available for each ~~patient~~ individual and the approach with which each cohort was ascertained. Correlation between different types of cerebral palsy was not explored and the health care-based cohort did not have parental samples to evaluate for variant inheritance since it was primarily made up of adult ~~patients~~. The authors note that this was an observational study and that no causal relationship between detected gene variants and phenotypes were established. Further research is required to understand and apply the clinical implications of the findings.

In a Clinical Utility Evaluation, Hayes (2021a) analyzed the evidence for the use of WES in children (younger than age 18) with suspected neurological disorders when standard testing was inconclusive. Studies reviewed indicated that changes in clinical management or therapies were available for 3% to 44% of individuals undergoing testing and when reported, there was an improvement in outcomes for some individuals tested, although a relatively small proportion (2% to 22%). Benefits such as the addition of closer surveillance or more diagnostic testing related to potential comorbidities, family planning and appropriate cascade testing for family members were also noted. In another Clinical Utility Evaluation (2021b, updated 2022) Hayes addressed the use of WES in individuals with a primary phenotype of intellectual disability, noting that current evidence is insufficient to support the use of this technology to assist with clinical management and/or improve outcomes for this specific population.

Hu et al. (2020) examined 60 pediatric patients from Beijing Children's Hospital suspected of having a genetic disorder including multiple congenital anomalies (MCA, n = 25), autism spectrum disorders (n = 4), development/intellectual disorders (DD/ID, n = 10), a combination of DD/ID and multiple congenital disorders (n = 6) and 15 with other phenotypes (e.g., congenital heart disease, short stature, recurrent infections). Trio whole exome sequencing (WES) and copy number variant (CNV) sequencing was performed to identify the diagnostic yield and clinical utility of parallel testing. A total of 37 pathogenic/likely pathogenic variants were found in 32 ~~patients~~ individuals (26 single nucleotide variants (SNV); 11 CNV). Of the SNVs identified, 65.4% were novel. Overall, the diagnosis rate was 53.3%. For the individuals that had positive results, 36.7% and 16.7% of positive results were diagnosed by WES and CNV, respectively. The diagnosis rates for ~~patients~~ individuals with DD/ID and/or MCA were greater than 50%. In addition to obtaining increased diagnosis rates for their cohort compared to traditional trio WES (36.7 to 53.3%) the authors concluded that they also achieved their secondary objectives of decreasing overall turnaround time by performing parallel testing (median 72 days) and helping physicians make easier choices about optimal testing regarding WES and CNV sequencing.

In a 2019 scoping review by the Neurodevelopmental Disorder (NDD) Exome Scoping Review Work Group, Srivastava et al. (included in the Hayes 2021a and 2022 Clinical Utility Evaluations) addressed exome sequencing (ES) for use in individuals with (NDDs). The study included a meta-analysis and subsequent consensus statement and the objective was to compare yield of ES with that of chromosome microarray analysis (CMA) in affected individuals. The study defined NDD as global DD, ID and/or autism spectrum disorder

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(ASD). A total of 30 articles addressing diagnostic yield in individuals with either NDD or NDD with associated conditions were analyzed. The yield of ES was 36% overall (31% for isolated NDD and 53% for NDD with associated conditions), which is substantially greater than previous studies focused on CMA (15-20%). The researchers conclude that the study showed consistently better performance of ES over CMA for evaluation of unexplained NDDs and recommend that ES should be used as a first-tier test. Noted limitations include focus on ID and/or ASD with potential exclusion of articles where phenotypes may have been less specific. Several of the included studies did not clearly define basis of ASD or ID/global DD, and certain studies with heterogeneous cohorts where number of individuals with NDD could not be determined were excluded, as well as studies including mtDNA sequencing. Authors Vissers (2017), Tarailo-Graovac (2016), Retterer, (2016) and Lee, (2014), previously cited in this policy, were included in the Srivastava systematic review and meta-analysis.

Groopman et al. (2019) studied the utility of WES in 3315 patients from two independent study cohorts with chronic kidney disease. A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events (AURORA) contributed 1128 patients, and 2773 patients came from a Columbia University Medical Center (CUMC) on end-stage renal disease who were recruited from 280 medical centers in 25 nations. For patients in the AURORA cohort, only broad categories and diagnostic codes for major clinical features were available, and detail clinical information from the EHR was available for the CUMC cohort. Most participants were over 21 years of age (92%) and of European ancestry (65%). WES provided a diagnostic result in 307 (9.3%) patients of 66 different genetic disorders. Diagnoses were found in all clinical categories, including congenital or cystic disease, and idiopathic nephropathies. Of those with a genetic diagnosis, 34 patients (1.6%) had medically actionable findings that included a change in renal management or referral to a subspecialty clinic.

The use of WES in the diagnostic workup of individuals with an idiopathic bleeding tendency was studied by Saes et al. (2019). A total of 87 patients at a mean age of 41 with a bleeding diathesis were analyzed using the Tosetto BAT score and standard diagnostic tests and divided into three groups: increased BAT with normal lab results (Group A), abnormal platelet count (Group B), or abnormal lab results without a definitive diagnosis (Group C). Patients were counseled by a clinical geneticist and consented to either a bleeding disorder gene panel only, or WES. All patients underwent WES, and for the targeted panel group, an in-silico panel was applied to select only known thrombosis and hemostasis genes. In the target panel analysis, fifteen patients (17%) were found to have a pathogenic variant in the targeted panel. Group A had the highest incidence of cases solved (24%), Group B had a 5% diagnostic yield, and Group C came in at 4%. Exome analysis was performed in 54 of the 80 unsolved cases. WES identified three VUS in candidate genes.

Gubbels et al (2019) performed rapid turnaround whole exome sequencing (rWES) on critically ill neonates based on phenotype-based selection. The prospective study cohort included infants less than 6 months of age from Boston Children's Hospital NICU with seizures, hypotonia, metabolic disorders and multiple congenital anomalies (MCA). Trio-based, rapid (< 7 day) WES was subsequently performed. Sixty percent (30/50) of infants met more than one phenotypic criterion and MCA was the most common phenotype seen in 37/50 (74%). Cardiac abnormalities, brain anomalies and dysmorphic features were also observed. The diagnostic yield for WES in this cohort was 58% (29/50). Of these 29 diagnoses, 27 involved a previously published gene and the other 2 were solved with pathogenic variants in novel disease genes. In 24/29 (82.7%) cases that were diagnosed by

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WES, families reported that the diagnosis made an impact for family and proband management. The most common effect of positive WES results on clinical management reported was influencing specialist referrals for further evaluations; medication and therapy changes, comfort care, early supportive care, reproductive decision-making and identification of other at-risk family members were also reported benefits. The authors stated that previous studies have shown that performing WES on all critically ill NICU patients has a lower diagnostic yield than when expert-based phenotypic criteria are used. Therefore, they recommended that the development of such criteria be validated to govern the use of genomic testing.

The BabySeq project is a pilot randomized trial within the Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT) study. NSIGHT is an NIH-funded consortium of four research programs designed to address questions and concerns about implementing routine WES into newborn care. Ceyhan-Birsoy et al. (2019) reports on their experience with the first 159 newborns analyzed in the BabySeq project, of which 127 were healthy newborns and 32 were ill and in the NICU. Fifteen newborns (ten healthy, five from the NICU) were found to be at risk for childhood onset diseases, none of which were anticipated from the known clinical or family histories. Five of these were in genes with a high penetrance rate, and included non-syndromic hearing loss, glomovenous malformations, KBG syndrome, biotinidase deficiency, and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Eleven genetic variants found in this sub-group were associated with moderate penetrance genetic disorders and were disclosed because of the possibility of early intervention. Examples included hypertrophic cardiomyopathy, aortic stenosis, atypical hemolytic-uremic syndrome, type I cystinuria and G6PD deficiency. Eighty-five newborns were found to have risks for adult-onset diseases, such as BRCA related cancer or Lynch syndrome. Only three newborns' parents chose to learn about the adult-onset disease risks. One hundred and forty of the newborns were found to be carriers of at least one autosomal recessive disorder. Pharmacogenetic test results were also returned and were limited to three genes felt by the BabySeq project to have the highest level of evidence for informing drug prescribing in the pediatric population; DPYD, TPMT and G6PD. Eight newborns had variants in these genes that could impact future care should the need for fluoropyrimidines or thiopurines arise. The infant with G6PD Deficiency was reported in the childhood onset disease section, as symptoms can be triggered by factors other than medications. Testing of parents was required and helpful in resolving results in thirteen cases. The authors concluded that this pilot study suggests that newborn WES may provide useful information beyond that currently available with routine newborn screening.

Cordoba et al. -(2018), included in the 2022 Hayes Clinical Utility Evaluation) performed a prospective study to determine the diagnostic yield and clinical utility of WES for patients with a probable neurogenetic disorder (n = 40). Family history, possible inheritance modes, disease characteristics and comorbidity studies were reviewed prior to WES for each study participant. The number and types of tests done prior to WES were also examined to compare the costs of diagnostic workups to WES as a stand-alone test. A diagnostic yield of 40% (95% CI, 24.8%-55.2%) for a diverse group of neurogenetic conditions was achieved. The positives in this group consisted of nine patients with autosomal dominant disease and seven with autosomal recessive. The researchers stated that it is noteworthy that 56% of the mutations were novel. The results led to altered treatment in 14 patients.

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Ambry Genetics, a genetic testing lab in California, described their retrospective experience of WES on 66 neonatal patients who were referred to their laboratory for testing due to clinical findings at birth suggestive of a genetic disease (Powis et al., 2018). Additional samples on parents or other family members were also sent when available. Rapid turnaround time requests had verbal results provided before Sanger sequencing confirmation, but all positive finding were verified with Sanger sequencing regardless. Secondary findings were available per ACMG guidelines when requested. The average turnaround time from test to report for the rapid analysis was 8 days, and with Sanger confirmation, 15 days. For non-rapid testing, the average turnaround time was 72 days. Over half of the patients reported no prenatal genetic testing, and 26 had a



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chromosome microarray and 4 had a post-natal single gene test or gene panel, but all were reported negative. Thirty-two patients had significant results from WES (48.5%). Fourteen had positive results in well characterized genes, eleven had likely positive results in well characterized genes, and six had VUS or uncertain gene/phenotype correlation in well characterized genes. One patient had a positive result in a novel candidate gene finding. Forty-eight patients consented to receiving secondary findings, and one patient was found to have a mutation in the low-density lipoprotein receptor *LDLR* gene. Changes in clinical management were reported by the authors on only one case; a male infant with low Apgar scores, limited spontaneous movement, and a fractured humerus. He tested negative for chromosome abnormalities, myotonic dystrophy, and spinal muscular atrophy. He had a complex family history.

Stark<sup>7</sup>, et al. (2018) explored the clinical utility of rapid whole exome sequencing (rWES) in acutely ill pediatric patients who were suspected to have a genetic disorder. Testing was performed on individual patients and did not include parents or other family members. A total of 40 patients met the enrollment criteria at two participating pediatric tertiary care centers in Melbourne, Australia between April 2016 and September 2017. Potential candidates were reviewed by a panel of study investigators, and a minimum of two medical geneticists had to agree that rWES was appropriate in order to enroll a patient. A phenotype driven list of candidate genes for prioritized analysis had to be provided by each clinician for their patient. Information on how the rWES impacted care was obtained from the patient's clinician. rWES provided a diagnosis in 21 (53%) of patients with a median time to diagnosis of 16 days. Most patients received the diagnosis during the hospital admission. rWES did better than biochemical testing for these patients. In one case, the diagnosis of aromatic L-amino acid decarboxylase (AADC) deficiency was made in 14 days with rWES, enabling immediate treatment, but it took 10 weeks for the biochemical results to be returned from the interstate lab. Additionally, rWES diagnosed short-chain enoyl-CoA hydratase (ECHS1) deficiency in a patient whose diagnostic urine pattern was obscured due to acute ketosis and acidosis. Only when the patient was stable and retested because of the rWES results did the urine metabolic screening become clear. Clinical management changed in 12 of the patients, including and included lifesaving treatment for a ventilator-dependent patient with a riboflavin transporter defect who ~~was ventilation dependent, but~~ was treated once the diagnosis was received and was discharged home shortly after. The authors noted that to successfully implement this program, it was necessary to develop a multi-disciplinary "rapid team" and create a whole system approach in order to overcome early barriers, such as delays in referral, patient assessments by the genetics team, complexities in patient genetic counseling, samples not clearly identified as "rapid," and the concern that time pressures impacting the quality of data analysis. The authors conclude that rWES is very promising and that developing the capability to deliver in pediatric and other settings requires substantial investment to optimize test performance and equity of access.

~~Trujillano et al. (2017) reported on the results of WES performed on 1,000~~ Nambot et al. (2018) reported on the effectiveness of regularly re-analyzing WES over a period of three years to address ongoing advances in bioinformatics approaches and updates to the medical literature. In a retrospective approach, the authors re-examined 416 WES tests that had been conducted in their clinic between June 2013 and June 2016. In the initial testing phase, 104 tests resulted in a diagnosis giving a diagnostic yield of 25%. There were 156 tests in the first two years of the study that did not provide a diagnosis or conclusive results and were reanalyzed. From this cohort, 24 new diagnoses were made with a yield of 15%. Half of the new diagnosis resulted from new information appearing in the literature, and bioinformatic pipeline updates resulting in reconsideration of misclassified variants and an improved ability to detect copy number variants. The other cases were resolved

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through collaboration with data sharing consortiums like the Matchmaker Exchange project, which uses case data to help researchers identify patients carrying variants in the same gene. The final overall yield of WES for this cohort, combining the initial results with the reanalysis, was 27.9%.

As part of the North Carolina Clinical Genomic Evaluation by Next-Generation Exome Sequencing Study (NCGENES), Haskell et al. (2018) used WES to determine a genetic diagnosis in 93 patients with NMD. Patients were categorized into three groups based on clinical findings: primarily neuropathy, primarily myopathy, or complex. After DNA extraction and WES, variants were filtered through three different gene lists in order to compare diagnostic yield between different lists. A neuropathy list of 199 genes implicated in neuropathy phenotypes, a myopathy list of 181 genes, and a list of 482 genes implicated in NMD were used. Variants were then categorized using the American College of Medical Genetics and Genomics (ACMG) standards on pathogenicity. The overall diagnostic yield of WES for pathogenic or likely pathogenic variants was 12.9%, and each gene list gave a different diagnostic yield. In some cases, family testing was performed to determine gene segregation and verify pathogenicity. The authors found that in patients with a clear neuropathy or myopathy, WES had the same diagnostic yield as the broader diagnostic test list. In patients with a complex phenotype, the broader list had the best diagnostic yield (9%) when compared to the neuropathy (4.9%) or myopathy (0%) diagnostic lists. Many of these patients had undergone muscle biopsy (42%), nerve conduction studies or electromyograms (86%), and genetic testing previously (68% overall and 20% had a multi-gene panel) and a definitive diagnosis had not been reached. The participant's biopsy, electrodiagnostic testing, and prior genetic results were reviewed by three independent specialist reviewers who categorized the testing as informative or non-informative in the context of WES results. Sixty-three percent of the prior testing was considered informative, meaning that it correlated with the pathogenic variant identified in WES as a neuropathy, myopathy, or a complex disorder. In two cases, WES identified molecular diagnoses that directly impacted medical treatment. One patient had been clinically diagnosed with a chronic inflammatory demyelinating polyneuropathy, but WES demonstrated that the genetic diagnosis of Spastic Ataxia of Charlevoix-Saguenay, so unnecessary immunotherapy was avoided. The second patient had been thought to have a hereditary spastic paraplegia, but the genetic diagnosis was confirmed as a form of dopa-responsive dystonia, and after dopa therapy was started, she regained the ability to walk without assistance. The authors concluded that introducing genome-scale sequencing into the clinical workflow earlier may shorten the diagnostic odyssey, minimize invasive testing, and provide potential opportunities for clinical and investigational therapeutics for patients with NMD.

Bardakjian et al. (2018) studied adult patients with neurological disorders who had been recommended to have genetic testing to determine the diagnostic yield of, and patient interest in, different types of tests in a real-world clinical setting. All patients were seen at a university-based specialty or neurogenetics clinic between January 2016 and April 2017 and were identified retrospectively through the electronic medical system. Overall, 377 patients were evaluated. The primary clinical indications for diagnostic genetic testing included ataxia, epilepsy, hereditary spastic paraparesis, leukodystrophy, memory loss, movement disorders, neuromuscular disease, and predictive testing due to a family history of disease, such as Huntington Disease. Genetic testing recommendations took place in a specialty clinic for 182 patients and 195 in the neurogenetics clinic. Eighty percent of patients had genetic testing completed. For those who chose not to have testing, 71 declined testing after genetic counseling, and 3 wanted to have testing, but it was not performed due to lack of insurance coverage. The highest rate of choosing not to test was in the category of patients referred for predictive



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testing for Huntington Disease. Age was not found to be a factor in accepting or declining testing. The overall diagnostic rate was 32% in the 303 people who completed testing. The yield was highest (50%) in targeted testing, where one or two genes were selected for testing based on clinical findings (n = 89). This category is followed by array comparative genome hybridization (aCGH) (45%) in 7 patients, followed by multigene panels (25%) in 155 patients, and exome testing (25%) in 52 patients. The authors reported that for individuals being worked up for dystonia, the use of a panel test reduced the time to diagnosis by 75%. In addition, the use of panel tests and WES increased the number of variants of uncertain significance (VUS). Using family segregation testing, de-identified genetic data-sharing through commercial platforms or academic consortia, the authors reduced the number of reportable VUS by one third but acknowledged this required the involvement of an expert clinician with the training and knowledge to resolve VUS.

The diagnostic utility of WES in adults with chronic kidney disease (CKD) was evaluated by Lata et al. (2018). Ninety-two individuals who were referred for analysis and workup due to CKD of unknown etiology or due to familial nephropathy or hypertension underwent WES. Overall a diagnosis was found in 24% of patients, including in 9 patients with CKD of unknown etiology. One BRCA2 mutation was found as an incidental finding, and the individual was diagnosed with breast cancer in a follow up appointment. Clinical management was altered in patients with a positive result and included a change in targeted surveillance, initiation of family screening to guide transplant donor selection, and changes in therapy.

Posey et al. (2016) performed a retrospective analysis of consecutive WES reports for adults from a diagnostic laboratory. Phenotype composition was determined using Human Phenotype Ontology terms. Molecular diagnoses were reported for 17.5% (85/486) of adults, lower than a primarily pediatric population (25.2%;  $p = 0.0003$ ); the diagnostic rate was higher (23.9%) in those 18 – 30 years of age compared to patients over 30 years (10.4%;  $p = 0.0001$ ). Dual Mendelian diagnoses contributed to 7% of diagnoses, revealing blended phenotypes. Diagnoses were more frequent among individuals with abnormalities of the nervous system, skeletal system, head/neck, and growth. Diagnostic rate was independent of family history information, and de novo mutations contributed to 61.4% of autosomal dominant diagnoses. This early WES experience in adults demonstrates molecular diagnoses in a substantial proportion of patients, informing clinical management, recurrence risk and recommendations for relatives. A positive family history was not predictive, consistent with molecular diagnoses often revealed by de novo events, informing the Mendelian basis of genetic disease in adults. Additional studies in WES sequencing are needed to validate its clinical utility. Trujillano et al. (2017) reported on the results of WES performed on 1000 consecutive cases with suspected Mendelian disorders from 54 countries (78.5% Middle East, 10.6% Europe, and 10.9% from rest of the world) referred for diagnostic WES between January 2014 and January 2016. Patients ranged between 1 month and 59 years, 92.4% were 15 years or younger, with 14.1% younger than 1 year and 39.4% 1 – 5 years of age. The cohort also included 23 prenatal cases (2.3%). Notably, 45.3% of the cases were from consanguineous families and 38.1% presented family history of the disease. Most cases (82.7%) were analyzed with a trio design (parents and index). They identified pathogenic or likely pathogenic variants in 307 families (30.7%). In further 253 families (25.3%) a variant of unknown significance, possibly explaining the clinical symptoms of the index patient was identified. WES enabled timely diagnosing of genetic diseases, validation of causality of specific genetic disorders of PTPN23, KCTD3, SCN3A, PPOX, FRMPD4, and SCN1B, and setting dual diagnoses by detecting two causative variants in distinct genes in the same patient. There was a better diagnostic yield in consanguineous families, in severe and in syndromic phenotypes. Based on these results,

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the authors recommend WES as a first-line diagnostic in all cases without a clear differential diagnosis.

Tan et al. (2017) conducted a prospective analysis of the utility of WES on consecutive patients presenting at the Victorian Clinical Genetics Services at the Royal Children's Children's Hospital, Melbourne, Australia in 2015. These patients were older than 2 years of age and were suspected of having a monogenic disorder. The children had not previously had diagnostic testing, such as a single gene or gene panel test, but may have had a non-diagnostic microarray. All participants underwent WES with a phenotype driven data analysis. Of 61 children assessed, 44 underwent WES. A diagnosis was achieved in 23 by sequencing the child alone. The diagnosis was unanticipated in 8 children, and altered clinical management in 6. The range of ages was 2-18 years old. The average length of "diagnostic odyssey" was 6 years, and prior to WES the average number of clinical tests was 19, with 4 genetics consults and 4 consults with other specialists. Fifty-nine children had undergone general anesthesia in order to perform a diagnostic test.

According to the ~~The authors, this study confirms~~ ~~hypothesize that WES at the first indication of a genetic disorder would have reduced the~~ clinical utility of WES in syndromic children ~~number of tests and~~ provides strong evidence to support its use early in the diagnostic trajectory ~~interventions, and provided an overall cost savings.~~

Posey et al. (2016) performed a retrospective analysis of consecutive WES reports for adults from a diagnostic laboratory. Phenotype composition was determined using Human Phenotype Ontology terms. Molecular diagnoses were reported for 17.5% (85/486) of adults, lower than a primarily pediatric population (25.2%; p = 0.0003); the diagnostic rate was higher (23.9%) in those 18 - 30 years of age compared to patients over 30 years (10.4%; p = 0.0001). Dual Mendelian diagnoses contributed to 7% of diagnoses, revealing blended phenotypes. Diagnoses were more frequent among individuals with abnormalities of the nervous system, skeletal system, head/neck, and growth. Diagnostic rate was independent of family history information, and de novo mutations contributed to 61.4% of autosomal dominant diagnoses. This early WES experience in adults demonstrates molecular diagnoses in a substantial proportion of patients, informing clinical management, recurrence risk and recommendations for relatives. A positive family history was not predictive, consistent with molecular diagnoses often revealed by de novo events, informing the Mendelian basis of genetic disease in adults. Additional studies in WES sequencing are needed to validate its clinical utility.

~~Visser~~Visser et al. (2017) of the Radboud University Medical Center in the Netherlands studied 150 consecutive patients presenting in the neurology clinic with non-acute neurological symptoms that were suspected to have a genetic origin, and compared the traditional work-up and testing paradigm to the use of WES. Both were conducted in parallel. The typical clinical approach gave a diagnostic yield of about 7%, whereas WES gave a diagnostic yield of about 29%. The authors highlighted the need for genetic counseling and tailored consent regarding incidental findings.

~~Tarailo~~Tarailo ~~Graovac~~ et al. (2016) combined deep clinical phenotyping (the comprehensive characterization of the discrete components of a patient's ~~patient's~~ clinical and biochemical phenotype) with WES analysis through a semiautomated bioinformatics pipeline in consecutively enrolled patients with intellectual developmental disorder and unexplained metabolic phenotypes. WES was performed on samples obtained from 47 probands. Of these patients, 6 were excluded, including 1 who withdrew from the study. The remaining 41 probands had been born to predominantly nonconsanguineous parents of European descent. In 37 probands, the investigators identified variants in 2 genes newly implicated in disease, 9 candidate genes, 22 known

~~genes with newly identified phenotypes, and 9 genes with expected phenotypes; in most of the genes, the variants were classified as either pathogenic or probably pathogenic. Complex phenotypes of patients in five families were explained by coexisting monogenic conditions. A diagnosis was obtained in 28 of 41 probands (68%) who were evaluated. A test of a targeted intervention was performed in 18 patients (44%). The authors concluded that deep phenotyping and WES in 41 probands with intellectual developmental disorder and unexplained metabolic abnormalities led to a diagnosis in 68%, the identification of 11 candidate genes newly implicated in neurometabolic disease, and a change in treatment beyond genetic counseling in 44%.~~

Stark et al. (2016) prospectively evaluated the diagnostic and clinical utility of singleton WES as a first-tier test in infants with suspected monogenic disease at a single pediatric tertiary center. This occurred in parallel with standard investigations, including single- or multigene panel sequencing when clinically indicated. The diagnosis rate, clinical utility, and impact on management of singleton WES were evaluated. Of 80 enrolled infants, 46 received a molecular genetic diagnosis through singleton WES (57.5%) compared with 11 (13.75%) who underwent standard investigations in the same patient group. Clinical management changed following exome diagnosis in 15 of 46 diagnosed participants (32.6%). Twelve relatives received a genetic diagnosis following cascade testing, and 28 couples were identified as being at high risk of recurrence in future pregnancies. The authors concluded that this prospective study provides strong evidence for increased diagnostic and clinical utility of singleton WES as a first-tier sequencing test for infants with a suspected monogenic disorder. Singleton WES outperformed standard care in terms of diagnosis rate and the benefits of a diagnosis, namely, impact on management of the child and clarification of reproductive risks for the extended family in a timely manner.

~~RettererRetterer et al. (2015) reported the diagnostic yield of WES in 3,040 consecutive cases at a single clinical laboratory. WES was performed for many different clinical indications and included the proband plus two or more family members in 76% of cases. The overall diagnostic yield of WES was 28.8%. The diagnostic yield was 23.6% in proband-only cases and 31.0% when three family members were analyzed. The highest yield was for patients who had disorders involving hearing (55%, n = 11), vision (47%, n = 60), the skeletal muscle system (40%, n = 43), the skeletal system (39%, n = 54), multiple congenital anomalies (36%, n = 729), skin (32%, n = 31), the central nervous system (31%, n = 1,082), and the cardiovascular system (28%, n = 54). Of 2,091 cases in which secondary findings were analyzed for 56 American College of Medical Genetics and Genomics-recommended genes, 6.2% (n = 129) had reportable pathogenic variants. In addition to cases with a definitive diagnosis, in 24.2% of cases a candidate gene was reported that may later be reclassified as being associated with a definitive diagnosis. According to the authors, analysis of trios significantly improves the diagnostic yield compared with proband-only testing for genetically heterogeneous disorders and facilitates identification of novel candidate genes.~~

~~ValenciaValencia et al. (2015) performed a retrospective review of the first 40 clinical cases to determine the performance characteristics of WES in a pediatric setting by describing patient cohort, calculating the diagnostic yield, and detailing the patients for whom clinical management was altered. Of these, genetic defects were identified in 12 (30%) patients, of which 47% of the mutations were previously unreported in the literature. Among the 12 patients with positive findings, seven had autosomal dominant disease and five had autosomal recessive disease. Ninety percent of the cohort opted to receive secondary findings and of those, secondary medical actionable results were returned in three cases. The diagnostic workup included a significant number of genetic~~

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~~tests with microarray and single-gene sequencing being the most popular tests. Genetic diagnosis from WES led to altered patient medical management in positive cases. The authors concluded that this review demonstrates the clinical utility of WES by establishing the clinical diagnostic rate and its impact on medical management in a large pediatric center. The cost-effectiveness of WES was demonstrated by ending the diagnostic odyssey in positive cases. According to the authors, in some cases it may be most cost-effective to directly perform WES.~~

~~Farwell et al. (2015) provided the results from the first 500 probands referred to a clinical laboratory for diagnostic exome sequencing. Family-based exome sequencing included WES followed by family inheritance-based model filtering, comprehensive medical review, familial cosegregation analysis, and analysis of novel genes. A positive or likely positive result in a characterized gene was identified in 30% of patients (152/500). A novel gene finding was identified in 7.5% of patients (31/416). The highest diagnostic rates were observed among patients with ataxia, multiple congenital anomalies, and epilepsy (44, 36, and 35%, respectively). Twenty three percent of positive findings were within genes characterized within the past 2 years. The diagnostic rate was significantly higher among families undergoing a trio (37%) as compared with a singleton (21%) whole-exome testing strategy. According to the authors, data demonstrate the utility of family-based exome sequencing and analysis to obtain the highest reported detection rate in an unselected clinical cohort, illustrating the utility of diagnostic exome sequencing as a transformative technology for the molecular diagnosis of genetic disease.~~

~~The Clinical Sequencing Exploratory Research Consortium (CSER) studied variant assessment between nine labs performing exome analysis and applying the ACMG and AMP sequence variant interpretation guidelines, and found that intra-lab concordance was 79%, but inter-lab concordance was only 34%. After consensus efforts, 70% concordance was achieved between labs, reflecting the continued subjectivity. Five percent of the discordant interpretations would impact clinical care (Green et al., 2016). In addition, because all genes are being analyzed simultaneously, an unexpected or incidental finding may be identified in the analysis that was outside of the clinical indication for the test (Richards et al., 2015). Novel variants may be discovered for the first time in the context of clinical care. Laboratories that perform WES are in the unique position of requiring detailed clinical information to interpret results and that may occasionally include testing of biological relatives (Richards et al., 2015).~~

Yang et al. (2014) performed clinical whole-exome sequencing and reported (1) the rate of molecular diagnosis among phenotypic groups, (2) the spectrum of genetic alterations contributing to disease, and (3) the prevalence of medically actionable incidental findings such as FBN1 mutations causing Marfan syndrome. This was an observational study of ~~2000~~ 2,000 consecutive patients with clinical WES analyzed between June 2012 and August 2014. WES tests were performed at a clinical ~~genetics'~~ genetics laboratory in the United States. Results were reported by clinical molecular geneticists certified by the American Board of Medical Genetics and Genomics. Tests were ordered by the ~~patient's~~ patient's physician. The patients were primarily pediatric (1756 [+1,756-(88%);%]; mean age, 6 years; 888 females [+44%], 1101 males [+55%]; and 11 fetuses [+1% gender unknown]), demonstrating diverse clinical manifestations most often including nervous system dysfunction such as developmental delay. A molecular diagnosis was reported for 504 patients (25.2%) with 58% of the diagnostic mutations not previously reported. Molecular diagnosis rates for each phenotypic category were 143/526 for the neurological group, 282/1147 for the neurological plus other organ systems group, 30/83 for the specific neurological group, and 49/244 for the non-neurological group. The

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Mendelian disease patterns of the 527 molecular diagnoses included 280 (53.1%) autosomal dominant, 181 (34.3%) autosomal recessives ~~recessive~~ (including 5 with uniparental disomy), 65 (12.3%) X-linked, and 1 (0.2%) mitochondrial. Of 504 patients with a molecular diagnosis, 23 (4.6%) had blended phenotypes resulting from 2 single gene defects. About 30% of the positive cases harbored mutations in disease genes reported since 2011. There were 95 medically actionable incidental findings in genes unrelated to the phenotype but with immediate implications for management in 92 patients (4.6%), including 59 patients (3%) with mutations in genes recommended for reporting by the American College of Medical Genetics and Genomics. The authors concluded that WES provided a potential molecular diagnosis for 25% of a large cohort of patients referred for evaluation of suspected genetic conditions, including detection of rare genetic events and new mutations contributing to disease. According to the authors, the yield of WES may offer advantages over traditional molecular diagnostic approaches in certain patients.

~~Lee et al. (2014) reported on initial clinical indications for clinical exome sequencing (CES) referrals and molecular diagnostic rates for different indications and for different test types. Clinical exome sequencing was performed on 814 consecutive patients with undiagnosed, suspected genetic conditions between January 2012 and August 2014. Clinical exome sequencing was conducted as trio-CES (both parents and their affected child sequenced simultaneously) to effectively detect de novo and compound heterozygous variants or as proband-CES (only the affected individual sequenced) when parental samples were not available. Of the 814 cases, the overall molecular diagnosis rate was 26%. The molecular diagnosis rate for trio-CES was 31% and 22% for proband-CES. In cases of developmental delay in children (< 5 years, n = 138), the molecular diagnosis rate was 41% for trio-CES cases and 9% for proband-CES cases. The significantly higher diagnostic yield of trio-CES was due to the identification of de novo and compound heterozygous variants. The authors concluded that in this sample of patients with undiagnosed, suspected genetic conditions, trio-CES was associated with higher molecular diagnostic yield than proband-CES or traditional molecular diagnostic methods.~~

~~Several other studies that evaluated WES have also reported that the diagnosis directly changed patient management (Soden et al., 2014; Srivastava et al., 2014; Zhu et al., 2015; Nolan and Carlson, 2016). WES results changed patient management in 45.3% to 76.9% of patients in 3 studies and 3.4% in 1 study. Changes to patient management included changes to drug or diet and referrals to other physicians/monitoring; results were reported to also change other family member's family planning and to guide their genetic testing. One study (Nolan and Carlson, 2016) reported on incidental findings (i.e., findings with clinical significance, such as a variant with a known disease-causing effect, but for a different condition than that being studied), which resulted in screening or monitoring of the patient (Hayes, 2016).~~

~~Nambot et al. (2017) reported on the effectiveness of regularly re-analyzing WES over a period of three years to address ongoing advances in bioinformatics approaches and updates to the medical literature. In a retrospective approach, the authors re-examined 416 WES tests that had been conducted in their clinic between June 2013 and June 2016. In the initial testing phase, 104 tests resulted in a diagnosis giving a diagnostic yield of 25%. There were 156 tests in the first two years of the study that did not provide a diagnosis or conclusive results and were reanalyzed. From this cohort, 24 new diagnoses were made with a yield of 15%. Half of the new diagnosis resulted from new information appearing in the literature, and bioinformatic pipeline updates resulting in reconsideration of misclassified variants and an improved ability to detect copy number variants. The other cases were resolved through collaboration with data sharing~~

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~~consortiums like the Matchmaker Exchange project, which uses case data to help researchers identify patients carrying variants in the same gene. The final overall yield of WES for this cohort, combining the initial results with the reanalysis, was 27.9%.~~

~~WES may result in false positives due to the difficulty in reading CG rich regions, and poor coverage depth (Meienberg et al., 2016). A comparison of standard next generation sequencing (NGS) techniques and WES demonstrated that > 98% of pathogenic variants are covered at depth adequate for detection (LaDuca et al., 2017).~~

~~For these reasons, it is critical that the ordering physician has specialty training and experience with these technologies and is prepared to work with the laboratory and interpret the results of such testing for their patient (Taber et al., 2014; Richards, et al., 2015; ACMG, 2012).~~

### WES: Pediatric (Cancer)

~~Zhang et al. (2015) studied the prevalence of cancer pre disposition germline mutations in children and adolescents with cancer in 1,120 patients under the age of 20. Whole exomes were sequenced in 456 patients and whole genomes were sequenced in 595, or both in 69. Results were analyzed in 565 genes, including 60 that are associated with autosomal dominant cancer syndromes. Genetic variant pathogenicity was determined by a team of experts who relied on peer reviewed literature, cancer and locus specific databases, computational predictions, and second hits identified in the participant tumor genome. This same variant calling approach was used to analyze data on 966 controls from the 1000 Genomes Project who were not known to have cancer and data from 733 children from an autism study. Overall, germline mutations were found in 95 children with cancer (8.5%), as compared to only 1.1% of the 1000 Genome Project and 0.6% of autism study controls. The mutations were most commonly found in TP53, APC, BRCA2, NF1, PMS2, RB1 and RUNX3. Eighteen patients also have variants in tumor suppressor genes. Of the 58 patients who had family history information available and a mutation in a predisposing dominant cancer gene, 40% had a significant family history of cancer.~~

~~The results of the German pilot study called 'Individualized Therapy for Relapsed Malignancies in Childhood' (INFORM) was reported on by Worst et al. in 2016. This was a precision medicine study utilizing tumor and blood whole exome, low coverage whole genome, and RNA sequencing, complemented with methylation and expression microarray analyses. The goal was to identify individualized therapies for children and adolescents diagnosed with a high risk relapsed/refractory cancer. Fifty seven patients from 20 centers were prospectively tested, and diagnoses included sarcomas (n = 25), brain tumors (n = 23), and other (n = 9).~~

~~Parsons et al. (2016) conducted a study to determine the prevalence of somatic and germline mutations in children with solid tumors. From August 2012 through June 2014, children with newly diagnosed and previously untreated central nervous system (CNS) and non CNS solid tumors were prospectively enrolled in the study at a large academic children's hospital. Blood and tumor samples underwent whole exome sequencing (WES) in a certified clinical laboratory with genetic results categorized by clinical relevance. A total of 150 children participated, with a mean age of 7 years, with 80 boys and 70 girls. Tumor samples were available for WES in 121 patients. In this group, somatic mutations with established clinical utility were found in 4 patients, and mutations with possible clinical utility were found in 29. CTNNE1 had the most mutations, followed by KIT, TSC2, BRAF, KRAS, and NRAS. Diagnostic germline mutations related to the child's clinical presentation was found in 150 patients and included 13 dominant mutations in known cancer susceptibility genes, including TP53, VHL, and BRCA1. One recessive liver~~



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~~disorder with liver cancer was identified in TJP2 and one renal cancer, CLCN5. Incidental findings were found in 8 patients. Nearly all patients (98%) had variants of unknown significance in known cancer genes, drug response genes, and genes known to be associated with recessive disorders.~~

There is insufficient evidence to support the use of WES for the prognosis or management of cancer. Further studies are needed to evaluate the clinical utility of WES for this indication.

To explore available options and assess impact related to genomic profiling in pediatric cancers, Summers et al. (2022) conducted a prospective precision medicine trial to identify various mutations in pediatric brain tumors, extracranial solid tumors and hematologic malignancies. A total of 127 tumors in 126 individuals were tested using WES of tumor and germline tissue and whole-transcriptome sequencing (RNA seq) of tumor tissue. The researchers found somatic alterations in 95.3% of tumor samples and detected cancer predisposition by using known pathogenic or likely pathogenic germline mutations in known cancer predisposition genes in 7.1% of participants. A new scoring system was developed for assessing impact of tumor and germline sequencing using cancer-related germline results, relevant genomic variations, recommendations for treatment and refined risk stratification/prognosis. In 85% of samples, at least one significant finding was recognized and in 65.1% of participants, recommendation to consider a targeted treatment agent was provided. Of those that received a recommendation, 24% actually received treatment using a molecularly targeted drug (16% of the total cohort). Based on this study's results, the authors assert that WES and RNA Seq was a feasible option and had clinical impact in high-risk children with cancer. Additional high quality studies with larger populations are needed.

Lee et al. (2021) performed a systematic review evaluating the published evidence addressing the use and efficacy of precision medicine in the context of pediatric oncology. Clinical trials and observational studies reporting clinical outcomes where molecular assays, including WES, were used to detect molecular targets that could aid in selection of targeted cancer drugs were the focus of this review. After applying exclusion criteria, 21 clinical trials and studies were included, comprising data from 1,408 children in 9 different countries. Ten of the included studies incorporated data on WES and two studies incorporated WGS data. Forty-six percent of children were found to have therapeutic targets, but only 27% of these actually received a targeted medication, reflecting probable barriers to access for targeted drugs. Treatment response was documented for 41.7% of those that received targeted medication (only 5.2% of the total sample group). Outcome reporting was inconsistent and limited the ability to compare with conventional treatments, thus inhibiting analysis of the clinical utility in this review. The authors recommend more clinical trials targeting molecular test results to targeted use of cancer medication and standardized outcomes to enhance understanding and support clinical utility for improved outcomes. Author Worst (2016), previously cited in this policy, was included in this systematic review.

Niu et al. (2019) performed a randomized controlled trial comparing clinical exome sequencing (CES) to usual care (UC) in the evaluation of clinical sensitivity and psychosocial outcomes for patients with inherited colorectal/polyposis (CRCP). Ninety-three participants completed surveys at 2 and 4 weeks after receipt of results and again at three-month intervals for one year. Approximately 94% of UC participants had multi-gene panels. Genetic findings confirmed the diagnosis of hereditary CRCP for 7.5% (7/93) vs. 5.4% (5/93) in the CES and UC arms, respectively (p = 0.28). Privacy concerns after



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receiving CRCP results were 0.38 in CES and 0.88 in UC ( $p = 0.05$ ). Alternatively, psychosocial outcomes, family communication, and healthcare resource utilization were comparable for both groups including 17.7% of patients with positive results planned to change insurance one month following initial return visit compared to 9.1% and 4.8% of patients with a variant of unknown significance or negative result, respectively ( $p = 0.09$ ). The authors indicated that the results of this study suggest that CES provides similar clinical benefits to multi-gene panels in the diagnosis of hereditary CRCP. According to the authors, future studies with larger sample sizes, that explore phenotypes for which there is a higher proportion of unknown genetic etiology and that include diverse and underserved patient populations to evaluate the psychosocial outcomes of CES using standardized, validated measures are needed.

The clinical impact of molecular profiling on pediatric tumors in children with refractory cancer was studied by Østrup et al. (2018) based on experiences in 2015 at the Center for Genomic Medicine, Rigshospitalet (Copenhagen, Denmark). Forty--six tumor samples, two bone marrow aspirates, three cerebral spinal fluid samples, and one archived tumor DNA from 48 children were analyzed by WES, RNA sequencing, transcriptome arrays, and single nucleotide polymorphism (SNP) arrays for mutation burden and to determine if actionable results could be found. Twenty patients had extracranial solid tumors and 25 had CNS tumors. Three patients were diagnosed with a hematological malignancy. Eleven of the 25 CNS tumors underwent additional DNA methylation profiling to obtain a second opinion on the diagnosis. At the time of the study, six patients were deceased. In 33 patients, actionable findings were identified which included 18 findings that helped make a final diagnosis, and 22 that allowed identification of potential treatment targets. Eleven findings had both a diagnostic and a treatment impact. Nine of the 33 findings were already known by prior histopathology tests. The highest yield for actionable findings was from WES (39%), followed by SNP array (37%) and RNA sequencing (21%). Clinical interventions based on these results were implemented in 11 of 44 patients, including 8 patients who received therapy based on the molecular profile. Six patients experienced direct benefit with improved response or stable disease. Four received compassionate use therapy. The authors commented that although 60% of the reports that went back to clinicians contained actionable findings, the clinicians encountered barriers to obtaining available or approved treatments which limited the utility of the advanced diagnostics. There are clinical trials available based on advanced molecular profiling, but the authors note that not all facilities have the infrastructure in place to provide comprehensive molecular profiling.

#### WES Prenatal

~~There are limited data on WES in prenatal genetic diagnostic testing. Fu et al. (2017) did sequential analysis involving karyotype, chromosome microarray (CMA), and then WES in a cohort of 3,949 structurally abnormal fetuses. Eighteen percent (720) fetuses had an abnormal karyotype. CMA analysis was performed on those with a normal karyotype (1,680) and 8% (168) had a pathogenic copy number variant. Of those with a normal karyotype and CMA analysis, 196 underwent WES, and 47 (24%) had a pathogenic variant identified that could potentially explain the phenotype; additionally, the incidence of variants of unknown significance (VUS) and secondary findings was 12% and 6%, respectively.~~

~~Aarabi et al. (2018) conducted a study of the utility of WES in prenatal cases with structural birth defects. Twenty fetuses with structural abnormalities with normal karyotype and chromosome microarray results underwent WES, as did their parents. Initial results using only prenatal ultrasound findings did not identify any pathogenic or likely pathogenic variants. WES results were later re-evaluated utilizing prenatal and post-natal phenotypes. Inclusion of the post natal phenotypes results in identifying~~

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~~pathogenic variants in 20% of cases including PORCN gene in a fetus with split hand/foot malformation, as well as reportable variants of uncertain significance in fetuses with postnatal muscle weakness and Adams Oliver syndrome. In one patient, post natal magnetic resonance imaging (MRI) identified the presence of holoprosencephaly. The case was referred for Sanger sequencing of related genes, and a 47 bp deletion was found in ZIC2 that was missed by WES. The authors suggest that incomplete fetal phenotyping limits the utility of WES, and that if prenatal WES is undertaken, re analysis of the data with additional postnatal phenotype information can be useful.~~

~~Further studies are needed to establish the clinical validity and clinical utility of WES in this setting.~~

#### ~~WES Adult (Non Cancer)~~

The Victorian Comprehensive Cancer Centre Alliance explored the feasibility of WES on solid tumor specimens that were proven to be malignant on biopsy in a pilot study (Lee et al., 2018). The study took place between March 2014 and March 2015, and 23 patients opted in for tumor WES. Targeted panel testing focusing on mutations in known hotspots for somatic mutations was performed. Fifteen samples passed the quality criteria for testing, and WES and targeted testing were successful on all samples. Of samples submitted, 65% success was achieved on formalin-fixed paraffin-embedded (FFPE) and 20% on fresh frozen (FF). A multi-disciplinary tumor board team reviewed the clinical relevance of identified variants and determined if they were actionable. WES identified somatic variants in 73% of cases, and targeted testing only found 53%. Clinical management changes were taken in 53% of cases that included referring patients to the familial cancer clinic and considering new therapies.

~~Bardakjian et al. (2018) studied adult patients with neurological disorders who had been recommended to have genetic testing to determine the diagnostic yield of, and patient interest in, different types of tests in a real world clinical setting. All patients were seen at a university based specialty or neurogenetics clinic between January 2016 and April 2017 and were identified retrospectively through the electronic medical system. Overall, 377 patients were evaluated. The primary clinical indications for diagnostic genetic testing included ataxia, epilepsy, hereditary spastic paraparesis, leukodystrophy, memory loss, movement disorders, neuromuscular disease, and predictive testing due to a family history of disease, such as Huntington Disease. Genetic testing recommendations took place in a specialty clinic for 182 patients and 195 in the neurogenetics clinic. Eighty percent of patients had genetic testing completed. For those who chose not to have testing, 71 declined testing after genetic counseling, and 3 wanted to have testing, but it was not performed due to lack of insurance coverage. The highest rate of choosing not to test was in the category of patients referred for predictive testing for Huntington Disease. Age was not found to be a factor in accepting or declining testing. The overall diagnostic rate was 32% in the 303 people who completed testing. The yield was highest (50%) in targeted testing, where one or two genes were selected for testing based on clinical findings (n = 89). This category is followed by array comparative genome hybridization (aCGH) (45%) in 7 patients, followed by multigene panels (25%) in 155 patients, and exome testing (25%) in 52 patients. The authors reported that for individuals being worked up for dystonia, the use of a panel test reduced the total cost of the diagnostic process by 30% by eliminating unnecessary tests like MRIs, and reduced the time to diagnosis by 75%. In addition, the use of panel tests and WES increased the number of variants of uncertain significance (VUS). Using family segregation testing, de-identified genetic data sharing through commercial platforms or academic consortia, the authors reduced the number of reportable VUS by one third, but~~

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~~acknowledged this required the involvement of an expert clinician with the training and knowledge to resolve VUS.~~

~~The diagnostic utility of WES in adults with chronic kidney disease (CKD) was evaluated by Lata et al. (2018). Ninety-two individuals who were referred for analysis and workup due to CKD of unknown etiology or due to familial nephropathy or hypertension underwent WES. Overall a diagnosis was found in 24% of patients, including in 9 patients with CKD of unknown etiology. One BRCA2 mutation was found as an incidental finding, and the individual was diagnosed with breast cancer in a follow up appointment. Clinical management was altered in patients with a positive result and included a change in targeted surveillance, initiation of family screening to guide transplant donor selection, and changes in therapy.~~

~~Posey et al. (2016) performed a retrospective analysis of consecutive WES reports for adults from a diagnostic laboratory. Phenotype composition was determined using Human Phenotype Ontology terms. Molecular diagnoses were reported for 17.5% (85/486) of adults, lower than a primarily pediatric population (25.2%;  $p = 0.0003$ ); the diagnostic rate was higher (23.9%) in those 18-30 years of age compared to patients over 30 years (10.4%;  $p = 0.0001$ ). Dual Mendelian diagnoses contributed to 7% of diagnoses, revealing blended phenotypes. Diagnoses were more frequent among individuals with abnormalities of the nervous system, skeletal system, head/neck, and growth. Diagnostic rate was independent of family history information, and de novo mutations contributed to 61.4% of autosomal dominant diagnoses. This early WES experience in adults demonstrates molecular diagnoses in a substantial proportion of patients, informing clinical management, recurrence risk and recommendations for relatives. A positive family history was not predictive, consistent with molecular diagnoses often revealed by de novo events, informing the Mendelian basis of genetic disease in adults. Additional studies in WES sequencing are needed to validate its clinical utility.~~

#### WES Adult Cancer

Nicolson et al. (2018) used WES to identify the genetic variants found in follicular thyroid cancer (FTC). They analyzed 39 tumors that were classified by subtype; 12 were minimally invasive (miFTC), 17 were encapsulated angioinvasive (eaFTC), and 10 were widely invasive (wiFTC). Samples were collected between 2002 and 2013. All samples were reviewed by a minimum of two independent pathologists to histopathological confirmation using the World Health Organization (WHO) 2017 guidelines. Hurthle cells were included, although differentiated by the WHO 2017 guidelines, because both Hurthle and conventional FTCs can exhibit invasive behavior. Samples underwent exome sequencing for a minimum 20X coverage, copy number variation analysis, and 13 of the samples were able to be tested for three common gene fusions found in FTC: PAX8-PPAR $\gamma$ , RET-PTC1, and RET-PTC3. Matched normal samples were collected from adjacent normal tissue or from white blood cell DNA. SciClone was used to detect clonal populations of tumor cells in each sample. Age, gender, tumor size (by largest diameter), and American Joint Committee on Cancer (AJCC) stage (7th and 8th editions), and genetic test results were assessed for association with invasive status. Most patients were female (67%), and the mean age was 55 years old. The medial tumor diameter was 3.6 cm and 92% had Stage I or Stage II disease. After surgery, patients were followed for disease progression for a median 5.8 years. The overall recurrence and disease progression rate was 15%. Overall, mutations in the RAS gene family were found in 20% of samples. TSHR mutations were identified in 4 tumors. DICER1, EIF1AX, KDM5C, NF1, PRDM1, PTEN, and TP53 were recurrently mutated in 2 samples each. The range of mutation burden in the tumors ranged from 1-44 variants per tumor. There were no statistically significant differences in mutation burden between subtypes. There were 55 germline variants found in potential cancer-associated genes, but none had been

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previously catalogued as a thyroid susceptibility gene. In general, the FTCs in this study had a general copy number gain. The most common gains were of 5q, 7p, and 12q. In the 13 samples that underwent fusion gene analysis, 1 was found to have the PAX8-PPARY fusion. When results were analyzed in the context of outcome, the total mutation burden, cancer driver burden, FTC driver burden and AJCC stage were all associated with worse prognosis. The authors' statistical analysis suggests that the genetic profile may be a strong prognostic factor independent of histopathology. More research is needed to determine if similar results could be obtained on less invasive biopsy specimens.

~~Most patients were female (67%)~~ The results of the German pilot study called 'Individualized Therapy for Relapsed Malignancies in Childhood' (INFORM) was reported on by Worst et al. in 2016. This was a precision medicine study utilizing tumor and blood whole-exome, low-coverage whole-genome, and RNA sequencing, complemented with methylation and expression microarray analyses. The goal was to identify individualized therapies for children and adolescents diagnosed with a high risk relapsed/refractory cancer. Fifty-seven patients from 20 centers were prospectively tested, and diagnoses included sarcomas (n = 25), brain tumors (n = 23), and other (n = 9).

Parsons et al. (2016) conducted a study to determine the prevalence of somatic and germline mutations in children with solid tumors. From August 2012 through June 2014, children with newly diagnosed and previously untreated central nervous system (CNS) and non-CNS solid tumors were prospectively enrolled in the study at a large academic children's hospital. Blood and tumor samples underwent whole exome sequencing (WES) in a certified clinical laboratory with genetic results categorized by clinical relevance. A total of 150 children participated, with a mean age of 7 years, with 80 boys and 70 girls. Tumor samples were available for WES in 121 patients. In this group, somatic mutations with established clinical utility were found in 4 patients, and mutations with possible clinical utility were found in 29. CTNNB1 had the most mutations, followed by KIT, TSC2, BRAF, KRAS, and NRAS. Diagnostic germline mutations related to the child's clinical presentation was found in 150 patients and included 13 dominant mutations in known cancer susceptibility genes, including TP53, VHL, and BRCA1. One recessive liver disorder with liver cancer was identified in TJP2 and one renal cancer, CLCN5. Incidental findings were found in 8 patients. Nearly all patients (98%) had variants of unknown significance in known cancer genes, drug response genes, and genes known to be associated with recessive disorders.

Zhang et al. (2015) studied the prevalence of cancer pre-disposition germline mutations in children and adolescents with cancer in 1,120 patients under the age of 20. Whole exomes were sequenced in 456 patients and whole genomes were sequenced in 595, or both in 69. Results were analyzed in 565 genes, including 60 that are associated with autosomal dominant cancer syndromes. Genetic variant pathogenicity was determined by a team of experts who relied on peer reviewed literature, cancer and locus specific databases, computational predictions, and second hits identified in the participant tumor genome. This same variant calling approach was used to analyze data on 966 controls from the 1000 Genomes Projects who were not known to have cancer and data from 733 children from an autism study. Overall, germline mutations were found in 95 children with cancer (8.5%), as compared to only 1.1% of 1000 Genome Project and 0.6% of autism study controls. The mutations were most commonly found in TP53, APC, BRCA2, NF1, PMS2, RB1 and RUNX3. Eighteen patients also have variants in tumor suppressor genes. Of the 58 patients who had family history information available and a mutation in a predisposing dominant cancer gene, 40% had a significant family history of cancer.

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Patients with metastatic and treatment-resistant cancer were prospectively enrolled at a single academic center for paired metastatic tumor and normal tissue WES during a 19-month period (Beltran et al., 2015). A comprehensive computational pipeline was used to detect point mutations, indels, and copy number alterations. Mutations were categorized as category 1, 2, or 3 on the basis of level of potential action; clinical reports were generated and discussed in precision tumor board. Patients (n = 97, with 154 tumor pairs) were observed for 7 to 25 months for correlation of molecular information with clinical response. Results showed that more than 90% of patients harbored actionable or biologically informative alterations, although treatment was guided by the information in only 5% of cases. This study highlights opportunities for future clinical trials regarding whole-exome sequencing in precision medicine.

Malhotra et al. (2014) evaluated whether there is evidence that WES improves outcomes for patients with cancer. Published evidence was evaluated using a methodology that combines the analytical validity, clinical validity, clinical utility and ethical, legal, and social implications (ACCE) model for genetic test evaluations with internationally accepted health technology assessment methodology. WES has been conducted most extensively (seven studies to date) in breast cancer patients, with fewer studies of other types of cancers (e.g., leukemia, prostate cancer, and ovarian cancer). Studies evaluating somatic alterations showed high intratumor and inter-tumor heterogeneity. In addition, both novel and previously implicated variants were identified. However, only three studies have shown potential for clinical utility of WES; whereby, variants identified through WES may determine response to drug treatment. The authors concluded that despite evidence for clinical validity of WES in cancers, clinical utility is very limited and needs to be further evaluated in large clinical studies.

#### **WES: ~~WES-Prenatal~~ Genetic Diagnosis or Screening**

There is insufficient evidence to support the use of WES for prenatal genetic diagnosis or screening. Further studies are needed to evaluate the clinical utility of ~~WES~~ for this indication.

Mellis et al. (2022) conducted a systematic review and meta-analysis to establish the diagnostic yield of ES when used for prenatal diagnosis of fetal structural anomalies after CMA is normal. The authors assessed 148 articles; 72 reports from 66 studies were included in this review, representing a total of 4,350 fetuses. Incremental diagnostic yield of ES over CMA/karyotyping was analyzed via meta-analysis as well as effects of case selection and impact on diagnostic yield by fetal phenotype. Pooled incremental yield of ES was 31% (95% confidence interval [CI] 26%-36%, p<010001). The diagnostic yield was significantly different between phenotypic sub-groups ranging from 2% for isolated increased nuchal translucency to 53% for isolated skeletal abnormalities and was substantially higher for cases that had been pre-selected for likelihood of monogenic etiology as compared to unselected cases ( 42% vs. 15%, p<010001). Based on these results, the researchers concluded that prenatal ES is able to provide a diagnosis in an additional 31% of fetuses with structural abnormalities after CMA and karyotyping has not provided a diagnosis. The diagnostic yield differs depending on the body system impacted and can be increased by specific pre-selection of cases after a multi-disciplinary review indicating likelihood of a monogenic cause. This review was limited by the high level of heterogeneity between the studies that were evaluated, impacting the level of comparison achievable. There was also variation in sample sizes and the method of analysis which likely impact diagnostic yield. Noted is the need for ongoing research on the clinical impact of prenatal ES to gain understanding regarding which pregnancies will benefit most and how to appropriately prioritize cases for testing and the challenges that exist for interpreting variants with incomplete and/or nonspecific information regarding phenotype.

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Authors Chen (2020), Deden (2020), Lord (2019), Petrovski (2019) Aarabi (2018), Fu (2018) and Normand (2018), previously cited in this policy, were included in this systematic review.

WES of the fetus and biological parents (trio testing) was used to analyze 500 pregnancies between the 11<sup>th</sup> and 31<sup>st</sup> week of gestation where abnormalities had been identified on fetal ultrasound (Gabriel et al. 2022). In most of the cases, negative non-invasive prenatal testing (NIPT), FISH rapid testing or chorionic short-time culture were obtained prior to exome analysis. After excluding maternal cell contamination, remaining variants were classified as per ACMG criteria and medically evaluated. In 37.8% of cases, pathogenic or likely pathogenic variants were identified that were determined to be causative to the fetal anomaly. This is comparable to the findings in postnatal trio exome studies. In 47.1% of the diagnosed fetuses, a heterozygous de novo variant was the cause of the anomaly and in 29.1% of the diagnosed fetuses, autosomal recessive diseases were identified. The average time to receive results was 17.8 days after the lab received the sample, although time to results decreased as the study progressed. The authors point out the large heterogeneity of the findings (pathogenic variants in 127 different genes) which highlights the importance of comprehensive exome diagnostics over panel diagnostics in fetal ultrasound anomalies. They assert that trio exome sequencing can be a useful tool in prenatal diagnostics but stress the importance of comprehensive, interdisciplinary counseling in conjunction with testing. Further high-quality studies using prenatal trio WES will be needed to establish clinical utility.

To further investigate the relationship of multisystem anomalies and the use of ES, Pauta et al. (2022) conducted a systematic review to ascertain the incremental diagnostic yield of ES in fetuses with multisystem structural anomalies (at least two in different anatomical systems) and negative CMA or karyotyping result. A total of 17 articles with data on ES diagnostic yield met inclusion criteria and were evaluated for this review including 694 fetuses with multisystem malformations. Subgroup analysis compared the diagnostic yield of the solo approach (fetus alone tested) and the trio approach (fetus and both biological parents tested). In 213 fetuses, a pathogenic or likely pathogenic variant was found that was potentially responsible for the fetal phenotype, representing an incremental yield of 33% (95% CI, 27-40%) for ES. Further assessment resulted in similar diagnostic yields of ES using either the solo approach (30%) or the trio approach (35%). Based on the results of this review, the authors conclude that potentially causative genes were identified when CMA or karyotyping was unsuccessful in approximately 1/3 of cases, with no meaningful differences between solo and trio approaches.

In a 2021 systematic review and meta-analysis, Pauta et al. sought to determine the diagnostic yield of ES in fetuses with recurrent fetal structural anomalies (where similar anomalies were found in consecutive pregnancies) with normal results of microarray and no family disease identified. The researchers pinpointed nine studies on diagnostic yield of ES including 140 fetuses with recurrent structural anomalies. Variants (either pathogenic or likely pathogenic) were found in 57 of the fetuses, representing an incremental diagnostic yield of 40% when using ES (95%CI: 26% to 54%). A recessive inheritance pattern was found in the majority of diseases identified (86%) and of these, 42% of variants were homozygous. Noted was that higher diagnostic yields appear to be associated with multisystem anomalies, as more than half the of positive results were in those fetuses with multisystem anomalies. The authors concluded that there is strong evidence that ES can be a powerful tool to uncover etiology of recurrent fetal malformations, especially monogenic syndromes, and they speculate that expansion from ES to genome sequencing (GS) will happen soon.



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A 2020 (updated 2022) Hayes Clinical Utility Evaluation found that the evidence supporting WES and WGS related to improvement of diagnosis and assistance with pregnancy and post-pregnancy management when abnormalities are detected by ultrasound or other testing is lacking. Large studies including outcome data and impact on clinical management are required to support clinical utility for the use of WES and WGS in the prenatal setting.

~~One hundred and five fetuses with congenital abnormalities detected by ultrasound who had negative testing results by quantitative fluorescent polymerase chain reaction and chromosomal microarray were evaluated by medical exome sequencing (MES). Chen et al. (2020) utilized trio-based MES to identify possible monogenic diseases in these fetuses. Known pathogenic coding and non coding regions of 4,000 disease causing genes were examined, and variants were categorized by the American College of Medical Genetics guidelines. Twelve phenotypic groups were established, and a confirmatory diagnosis was made in 19% (20/105) of fetuses. Twenty-one pathogenic or likely pathogenic variants were discovered in 14 genes. Diagnostic yield of genetic variants differed between the phenotypic groups with the highest yield being seen for cardiovascular abnormalities (44%); 38% and 25% of skeletal/limb abnormalities revealed and 25% of brain anomalies, respectively, revealed pathogenic variants. VUS were detected in 12 fetuses. The authors surmised that MES identifies the genetic cause of structural congenital anomalies and further aids in pregnancy management and counseling. The authors indicated that although prenatal MES is promising, more research is needed to address challenges related to clinical utility, indication for the test, interpretation of pathogenicity, variants of uncertain significance and report of incidental or secondary findings.~~

~~Deden et al. (2020) conducted an observational examination of 54 patients referred for rapid whole exome sequencing (rWES) to determine the diagnostic yield and clinical utility of trio-based rWES for fetuses diagnosed by ultrasound with multiple congenital anomalies. Common ultrasound findings reported in this study included skeletal dysplasia (n = 20), intracerebral structural abnormalities (n = 7), and multiple congenital abnormalities (n = 17). Definitive diagnosis was confirmed in 18/54 cases (33%). Skeletal dysplasia findings revealed the majority of pathogenic variants reported (n = 11) followed by four cases with multiple congenital anomalies and three cases with intracerebral abnormalities. Physicians for 37/54 patients completed surveys and reported that 68% of the participants experienced significant impacts to their clinical decision making. This study concluded that rWES enhanced prenatal diagnosis of fetuses with congenital anomalies and significantly influenced parental prenatal clinical decision making. According to the authors, this observation may be the result of the study's relatively small cohort size or clinical representativeness, thereby limiting further firm conclusions from these observations.~~

~~Lord et al. (2019) reported on a prospective cohort study involving 34 fetal medicine units in England and Scotland. WES was used to evaluate the presence or absence of causative genetic variants in fetuses with structural anomalies identified on ultrasound after 11 weeks gestation. Women were included if they chose invasive testing, karyotyping or chromosome microarray testing was negative for aneuploidy or other copy number variants, and the father consented to being tested as well to enable a trio analysis. The study took place between October 2014 and June 2017, and clinical outcomes were collected until March 2018. In all, 610 fetuses and 1202 parent samples were tested. A diagnostic result was found in 52 (8.5%), and a VUS with potential clinical usefulness was found in 24 (3.9%). In 143 fetuses with multisystem anomalies, the diagnostic yield was 15.4%, in those with cardiac anomalies it was 11.1%, and 15.4% in those with skeletal anomalies. The lowest diagnostic yield was in those with an isolated nuchal thickness (3.2%).~~



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~~Although WES improved the identification of genetic disorders in fetuses with structural anomalies, the authors conclude that careful consideration should be given to which cases are selected.~~

~~A prospective analysis was undertaken by Petrovski et al. (2019) at Columbia University Carmen and John Thain Center for Prenatal Pediatrics in New York. Between April 2015 and April 2017, 517 pregnancies with structural abnormalities identified by ultrasound were offered the opportunity to participate in a clinical utility study of trio WES. Eligible participants had a negative fetal karyotype or chromosome microarray. Overall a total of 234 trios consented and had good quality samples and completed testing. The diagnostic yield was 10% (24 pregnancies). There were also 46 pregnancies with variants that were likely pathogenic, but there was insufficient evidence to consider the result truly pathogenic. Nine of the twenty-four pregnancies with a diagnosis were found to have a diagnosis that impacted future reproductive counseling. At the time of publication, two carrier couples had sought CVS in a subsequent pregnancy, and one is seeking preimplantation diagnosis. One family that had a suspected likely pathogenic variant had a second affected pregnancy before enough information was available in the medical literature to call the variant pathogenic, highlighting the difficult balance between over and under reporting VUS. The authors concluded that fetal exome analysis is feasible but is complex and requires the collaboration of a multidisciplinary team.~~

~~The clinical utility of fetal exome sequencing was retrospectively reviewed by Normand et al. (2018) for 146 consecutive fetal exomes performed at the Baylor Genetics clinical diagnostic laboratory. Patients were included if reported between 2012 and 2017, where the fetal sample was derived from an amniocentesis, chorionic villi sampling (CVS), or a cordocentesis, or was a product of conception and the fetus had a confirmed anomaly on imaging or autopsy. A molecular diagnosis was found in 46 (32%) of fetuses. The number anomalies that were noted in the fetus was correlated with the likelihood of a genetic diagnosis. Excluding tissue culture time, the average turn-around time for a report was 14 days. The diagnostic rate did not differ between those with a contributing family history and a spontaneous case. Medical management changes were available in 14 cases. In four cases, medical management was altered by altering the neonatal care pregnancy termination plans. The remaining ten cases resulted in altered reproductive planning for the future, such as choosing preimplantation genetic diagnosis. The authors concluded that more research is needed overall on the clinical utility of fetal exome testing but select cases may be appropriate for WES under the guidance of an experience multi-disciplinary team.~~

~~Aarabi et al. (2018) conducted a study of the utility of WES in prenatal cases with structural birth defects. Twenty fetuses with structural abnormalities with normal karyotype and chromosome microarray results underwent WES, as did their parents. Initial results using only prenatal ultrasound findings did not identify any pathogenic or likely pathogenic variants. WES results were later re-evaluated utilizing prenatal and post-natal phenotypes. Inclusion of the post-natal phenotypes results in identifying pathogenic variants in 20% of cases including PORCN gene in a fetus with split-hand/foot malformation, as well as reportable variants of uncertain significance in fetuses with postnatal muscle weakness and Adams-Oliver syndrome. In one patient, post-natal magnetic resonance imaging (MRI) identified the presence of holoprosencephaly. The case was referred for Sanger sequencing of related genes, and a 47 bp deletion was found in ZIC2 that was missed by WES. The authors suggest that incomplete fetal phenotyping limits the utility of WES, and that if prenatal WES is undertaken, re-analysis of the data with additional postnatal phenotype information can be useful. Further studies are needed to establish the clinical validity and clinical utility of WES in this setting.~~

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Fu et al. (2017) did sequential analysis involving karyotype, chromosome microarray (CMA), and then WES in a cohort of 3949 structurally abnormal fetuses. Eighteen percent (720) fetuses had an abnormal karyotype. CMA analysis was performed on those with a normal karyotype (1680) and 8% (168) had a pathogenic copy number variant. Of those with a normal karyotype and CMA analysis, 196 underwent WES, and 47 (24%) had a pathogenic variant identified that could potentially explain the phenotype; additionally, the incidence of variants of unknown significance (VUS) and secondary findings was 12% and 6%, respectively.

#### WES Adult (Non-Cancer)

Groopman et al. (2019) studied the utility of WES in 3315 patients from two independent study cohorts with chronic kidney disease. A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events (AURORA) contributed 1128 patients, and 2773 patients came from a Columbia University Medical Center (CUMC) on end stage renal disease who were recruited from 280 medical centers in 25 nations. For patients in the AURORA cohort, only broad categories and diagnostic codes for major clinical features were available, and detail clinical information from the EHR was available for the CUMC cohort. Most participants were over 21 years of age (92%) and of European ancestry (65%). WES provided a diagnostic result in 307 (9.3%) patients of 66 different genetic disorders. Diagnoses were found in all clinical categories, including congenital or cystic disease, and idiopathic nephropathies. Of those with a genetic diagnosis, 34 patients (1.6%) had medically actionable findings that included a change in renal management or referral to a subspecialty clinic.

The use of WES in the diagnostic workup of individuals with an idiopathic bleeding tendency was studied by Saes et al. (2019). A total of 87 patients at a mean age of 41 with a bleeding diathesis were analyzed using the Tosetto BAT score and standard diagnostic tests and divided into three groups: increased BAT with normal lab results (Group A), abnormal platelet count (Group B), or abnormal lab results without a definitive diagnosis (Group C). Patients were counseled by a clinical geneticist and consented to either a bleeding disorder gene panel only, or WES. All patients underwent WES, and for the targeted panel group, an in-silico panel was applied to select only known thrombosis and hemostasis genes. In the target panel analysis, fifteen patients (17%) were found to have a pathogenic variant in the targeted panel. Group A had the highest incidence of cases solved (24%), Group B had a 5% diagnostic yield, and Group C came in at 4%. Exome analysis was performed in 54 of the 80 unsolved cases. WES identified three VUS in candidate genes.

Cordoba et al. (2018) performed a prospective study to determine the diagnostic yield and clinical utility of WES for patients with a probable neurogenetic disorder (n = 40). Family history, possible inheritance modes, disease characteristics and comorbidity studies were reviewed prior to WES for each study participant. The number and types of tests done prior to WES were also examined to compare the costs of diagnostic workups to WES as a stand-alone test. A diagnostic yield of 40% (95% CI, 24.8%-55.2%) for a diverse group of neurogenetic conditions was achieved. The positives in this group consisted of nine patients with autosomal dominant disease and seven with autosomal recessive. The researchers stated that it is noteworthy that 56% of the mutations were novel. The results led to altered treatment in 14 patients.

Ewans et al. (2018) performed whole exome sequencing (WES) on a cohort of 54 patients from an Australian clinical genetics unit to determine the clinical validity of WES for

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individuals with no known diagnosis. After 12 months, re-evaluation was performed to determine the extent of increase in diagnostic yield. Study participants met the following criteria: symptoms suggestive of a monogenic condition; family history suggestive of Mendelian inheritance; previously negative test results; informed consent for genetic testing. The phenotypes in this cohort consisted of 49% syndromic intellectual disability (s-ID), 13% skeletal defects, 11% hematological, 8% visual, 5% neurological, 3% metabolic and 3% syndromic. Pediatric cases comprised two-thirds of this cohort. Pathogenic variants were reviewed by the American College of Medical Genetics and a genetic diagnosis was made in 30% of probands. Repeat WES analysis after 12 months increased the diagnostic yield to 41%. Despite WES data reanalysis, 60% of this cohort remained undiagnosed. The authors indicated that they expect that the annual reanalysis of WES data will continue to provide additional diagnoses with an ongoing increased yield.

As part of the North Carolina Clinical Genomic Evaluation by Next Generation Exome Sequencing Study (NCGENES), Haskell et al. (2018) used WES to determine a genetic diagnosis in 93 patients with NMD. Patients were categorized into three groups based on clinical findings: primarily neuropathy, primarily myopathy, or complex. After DNA extraction and WES, variants were filtered through three different gene lists in order to compare diagnostic yield between different lists. A neuropathy list of 199 genes implicated in neuropathy phenotypes, a myopathy list of 181 genes, and a list of 482 genes implicated in NMD were used. Variants were then categorized using the American College of Medical Genetics and Genomics (ACMG) standards on pathogenicity. The overall diagnostic yield of WES for pathogenic or likely pathogenic variants was 12.9%, and each gene list gave a different diagnostic yield. In some cases, family testing was performed to determine gene segregation and verify pathogenicity. The authors found that in patients with a clear neuropathy or myopathy, WES had the same diagnostic yield as the broader diagnostic test list. In patients with a complex phenotype, the broader list had the best diagnostic yield (9%) when compared to the neuropathy (4.9%) or myopathy (0%) diagnostic lists. Many of these patients had undergone muscle biopsy (42%), nerve conduction studies or electromyograms (86%), and genetic testing previously (68% overall and 20% had a multi gene panel) and a definitive diagnosis had not been reached. The participant's biopsy, electrodiagnostic testing, and prior genetic results were reviewed by three independent specialist reviewers who categorized the testing as informative or non-informative in the context of WES results. Sixty three percent of the prior testing was considered informative, meaning that it correlated with the pathogenic variant identified in WES as a neuropathy, myopathy, or a complex disorder. In two cases, WES identified molecular diagnoses that directly impacted medical treatment. One patient had been clinically diagnosed with a chronic inflammatory demyelinating polyneuropathy, but WES demonstrated that the genetic diagnosis of Spastic Ataxia of Charlevoix-Saguenay, so unnecessary immunotherapy was avoided. The second patient had been thought to have a hereditary spastic paraplegia, but the genetic diagnosis was confirmed as a form of dopa-responsive dystonia, and after dopa therapy was started, she regained the ability to walk without assistance. The authors concluded that introducing genome scale sequencing into the clinical workflow earlier may shorten the diagnostic odyssey, minimize invasive testing, and provide potential opportunities for clinical and investigational therapeutics for patients with NMD.

Bardakjian et al. (2018) studied adult patients with neurological disorders who had been recommended to have genetic testing to determine the diagnostic yield of, and patient interest in, different types of tests in a real world clinical setting. All patients were seen at a university based specialty or neurogenetics clinic between January 2016 and April 2017 and were identified retrospectively through the electronic medical system.

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~~Overall, 377 patients were evaluated. The primary clinical indications for diagnostic genetic testing included ataxia, epilepsy, hereditary spastic paraparesis, leukodystrophy, memory loss, movement disorders, neuromuscular disease, and predictive testing due to a family history of disease, such as Huntington Disease. Genetic testing recommendations took place in a specialty clinic for 182 patients and 195 in the neurogenetics clinic. Eighty percent of patients had genetic testing completed. For those who chose not to have testing, 71 declined testing after genetic counseling, and 3 wanted to have testing, but it was not performed due to lack of insurance coverage. The highest rate of choosing not to test was in the category of patients referred for predictive testing for Huntington Disease. Age was not found to be a factor in accepting or declining testing. The overall diagnostic rate was 32% in the 303 people who completed testing. The yield was highest (50%) in targeted testing, where one or two genes were selected for testing based on clinical findings (n = 89). This category is followed by array comparative genome hybridization (aCGH) (45%) in 7 patients, followed by multigene panels (25%) in 155 patients, and exome testing (25%) in 52 patients. The authors reported that for individuals being worked up for dystonia, the use of a panel test reduced the time to diagnosis by 75%. In addition, the use of panel tests and WES increased the number of variants of uncertain significance (VUS). Using family segregation testing, de identified genetic data sharing through commercial platforms or academic consortia, the authors reduced the number of reportable VUS by one third but acknowledged this required the involvement of an expert clinician with the training and knowledge to resolve VUS.~~

~~The diagnostic utility of WES in adults with chronic kidney disease (CKD) was evaluated by Lata et al. (2018). Ninety two individuals who were referred for analysis and workup due to CKD of unknown etiology or due to familial nephropathy or hypertension underwent WES. Overall a diagnosis was found in 24% of patients, including in 9 patients with CKD of unknown etiology. One BRCA2 mutation was found as an incidental finding, and the individual was diagnosed with breast cancer in a follow up appointment. Clinical management was altered in patients with a positive result and included a change in targeted surveillance, initiation of family screening to guide transplant donor selection, and changes in therapy.~~

~~Posey et al. (2016) performed a retrospective analysis of consecutive WES reports for adults from a diagnostic laboratory. Phenotype composition was determined using Human Phenotype Ontology terms. Molecular diagnoses were reported for 17.5% (85/486) of adults, lower than a primarily pediatric population (25.2%; p = 0.0003); the diagnostic rate was higher (23.9%) in those 18–30 years of age compared to patients over 30 years (10.4%; p = 0.0001). Dual Mendelian diagnoses contributed to 7% of diagnoses, revealing blended phenotypes. Diagnoses were more frequent among individuals with abnormalities of the nervous system, skeletal system, head/neck, and growth. Diagnostic rate was independent of family history information, and de novo mutations contributed to 61.4% of autosomal dominant diagnoses. This early WES experience in adults demonstrates molecular diagnoses in a substantial proportion of patients, informing clinical management, recurrence risk and recommendations for relatives. A positive family history was not predictive, consistent with molecular diagnoses often revealed by de novo events, informing the Mendelian basis of genetic disease in adults. Additional studies in WES sequencing are needed to validate its clinical utility.~~

#### WES Adult Cancer

~~There is insufficient evidence to support the use of WES for the prognosis or management of cancer. Further studies are needed to evaluate the clinical utility of WES for this indication.~~

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~~Niu et al. (2019) performed a randomized controlled trial comparing clinical exome sequencing (CES) to usual care (UC) in the evaluation of clinical sensitivity and psychosocial outcomes for patients with inherited colorectal/polypsis (CRCP). Ninety-three participants completed surveys at 2 and 4 weeks after receipt of results and again at three month intervals for one year. Approximately 94% of UC participants had multi-gene panels. Genetic findings confirmed the diagnosis of hereditary CRCP for 7.5% (7/93) vs. 5.4% (5/93) in the CES and UC arms, respectively ( $p = 0.20$ ). Privacy concerns after receiving CRCP results were 0.38 in CES and 0.88 in UC ( $p = 0.05$ ). Alternatively, psychosocial outcomes, family communication, and healthcare resource utilization were comparable for both groups including 17.7% of patients with positive results planned to change insurance one month following initial return visit compared to 9.1% and 4.8% of patients with a variant of unknown significance or negative result, respectively ( $p = 0.09$ ). The authors indicated that the results of this study suggest that CES provides similar clinical benefits to multi-gene panels in the diagnosis of hereditary CRCP. According to the authors, future studies with larger sample sizes, that explore phenotypes for which there is a higher proportion of unknown genetic etiology and that include diverse and underserved patient populations to evaluate the psychosocial outcomes of CES using standardized, validated measures are needed.~~

~~The Victorian Comprehensive Cancer Centre Alliance explored the feasibility of WES on solid tumor specimens that were proven to be malignant on biopsy in a pilot study (Lee et al., 2018). The study took place between March 2014 and March 2015, and 23 patients opted in for tumor WES. Targeted panel testing focusing on mutations in known hotspots for somatic mutations was performed. Fifteen samples passed the quality criteria for testing, and WES and targeted testing were successful on all samples. Of samples submitted, 65% success was achieved on formalin-fixed paraffin-embedded (FFPE) and 20% on fresh frozen (FF). A multi-disciplinary tumor board team reviewed the clinical relevance of identified variants and determined if they were actionable. WES identified somatic variants in 73% of cases, and targeted testing only found 53%. Clinical management changes were taken in 53% of cases that included referring patients to the familial cancer clinic and considering new therapies.~~

~~Nicolson et al. (2018) used WES to identify the genetic variants found in follicular thyroid cancer (FTC). They analyzed 39 tumors that were classified by subtype; 12 were minimally invasive (miFTC), 17 were encapsulated angioinvasive (eaFTC), and 10 were widely invasive (wiFTC). Samples were collected between 2002 and 2013. All samples were reviewed by a minimum of two independent pathologists to histopathological confirmation using the World Health Organization (WHO) 2017 guidelines. Hurthle cells were included, although differentiated by the WHO 2017 guidelines, because both Hurthle and conventional FTCs can exhibit invasive behavior. Samples underwent exome sequencing for a minimum 20X coverage, copy number variation analysis, and 13 of the samples were able to be tested for three common gene fusions found in FTC: *PAX8-PPAR $\gamma$* , *RET-PTC1*, and *RET-PTC3*. Matched normal samples were collected from adjacent normal tissue or from white blood cell DNA. SeiClone was used to detect clonal populations of tumor cells in each sample. Age, gender, tumor size (by largest diameter), and American Joint Committee on Cancer (AJCC) stage (7th and 8th editions), and genetic test results were assessed for association with invasive status. Most patients were female (67%), and the mean age was 55 years old. The medial tumor diameter was 3.6 cm and 92% had Stage I or Stage II disease. After surgery, patients were followed for disease progression for a median 5.8 years. The overall recurrence and disease progression rate was 15%. Overall, mutations in the RAS gene family were found in 20% of samples. TSHR mutations were identified in 4 tumors. *DICER1*, *E1F1AX*, *KDM5C*, *NF1*, *PRDM1*, *PTEN*, and *TP53* were recurrently mutated in 2 samples each. The~~

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range of mutation burden in the tumors ranged from 1-44 variants per tumor. There were no statistically significant differences in mutation burden between subtypes. There were 55 germline variants found in potential cancer-associated genes, but none had been previously catalogued as a thyroid susceptibility gene. In general, the FTCs in this study had a general copy number gain. The most common gains were of 5q, 7p, and 12q. In the 13 samples that underwent fusion gene analysis, 1 was found to have the *PAX8-PPAR $\gamma$*  fusion. When results were analyzed in the context of outcome, the total mutation burden, cancer driver burden, FTC driver burden and AJCC stage were all associated with worse prognosis. The authors' statistical analysis suggests that the genetic profile may be a strong prognostic factor independent of histopathology. More research is needed to determine if similar results could be obtained on less invasive biopsy specimens.

Patients with metastatic and treatment resistant cancer were prospectively enrolled at a single academic center for paired metastatic tumor and normal tissue WES during a 19-month period (Beltran et al., 2015). A comprehensive computational pipeline was used to detect point mutations, indels, and copy number alterations. Mutations were categorized as category 1, 2, or 3 on the basis of level of potential action; clinical reports were generated and discussed in precision tumor board. Patients (n = 97, with 154 tumor pairs) were observed for 7 to 25 months for correlation of molecular information with clinical response. Results showed that more than 90% of patients harbored actionable or biologically informative alterations, although treatment was guided by the information in only 5% of cases. This study highlights opportunities for future clinical trials regarding whole exome sequencing in precision medicine.

Malhotra et al. (2014) evaluated whether there is evidence that WES improves outcomes for patients with cancer. Published evidence was evaluated using a methodology that combines the analytical validity, clinical validity, clinical utility and ethical, legal, and social implications (ACCE) model for genetic test evaluations with internationally accepted health technology assessment methodology. WES has been conducted most extensively (seven studies to date) in breast cancer patients, with fewer studies of other types of cancers (e.g., leukemia, prostate cancer, and ovarian cancer). Studies evaluating somatic alterations showed high intratumor and inter-tumor heterogeneity. In addition, both novel and previously implicated variants were identified. However, only three studies have shown potential for clinical utility of WES, whereby, variants identified through WES may determine response to drug treatment. The authors concluded that despite evidence for clinical validity of WES in cancers, clinical utility is very limited and needs to be further evaluated in large clinical studies.

In a breast cancer study, follow-up analyses showed enrichment of *GATA3* variants (identified by WES) in samples showing a decline in Ki-67 levels, which is a marker for response to aromatase inhibitor treatment. This association suggests that presence of *GATA3* variants may be a predictive marker to identify individuals who will respond to treatment with aromatase inhibitors (Ellis et al., 2012). A second study showed that somatic hyper variation detected through WES not only was a predictive factor for determining platinum-based chemotherapy response in ovarian cancer treatment, but was also statistically significantly associated with longer overall survival and progression-free survival (Sohn et al., 2012). Another WES study by Tzoneva et al. (2013) identified *NT5C2* variants that were associated with AML relapse even when receiving treatment, and early relapse compared to late relapse, suggesting *NT5C2* may be a potential marker to identify individuals who may experience AML relapse despite chemotherapy treatment. While these 3 studies suggest potential for clinical utility, they need to be validated in larger clinical studies.



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## WES: Reanalysis

The Undiagnosed Rare Disease Program of Catalonia (URD-Cat) project (Bullich et al., 2022) systematically reanalyzed data including genomic panels, ES and GS along with standardized phenotypes from 543 individuals in 323 families with undiagnosed neurologic diseases. Specifically, relatedness, consanguinity, runs of homozygosity, single-nucleotide variants, insertions and deletions and copy number variants were reinvestigated in the existing data. Collaborative interpretation was performed using a customized Genome-Phenome Analysis Platform (GPAP). This reanalysis resulted in a diagnosis for 20.7% of individuals, 1.8% of whom were diagnosed after the generation of additional genomic data used to pinpoint a second pathogenic heterozygous variant. The study results indicated a significantly higher diagnostic rate for family-based exome and genome reanalysis when compared with individual panels. Recent gene-disease associations were responsible for the majority of new diagnoses (50.8%). Other factors responsible for ability to reach a diagnosis were additional/improved bioinformatic analysis (19.7%) and standardized phenotyping data in the platform used (18%). Overall, this reanalysis led to a diagnosis in 67 individuals, which, according to their referring clinicians, would enable affected individuals to receive better medical management, enable genetic counseling for parents/family members, and to lead to potential diagnoses in other affected family members. The authors conclude that use of the GPAP tool was key to efficient reanalysis of genomic information and data sharing.

In an effort to determine the efficiency of distinct strategies for reanalysis of negative ES reports in undiagnosed children with neurological conditions, Schobers et al. (2022) executed a systematic study. The study included 103 genetically undiagnosed children who underwent reanalysis, including ES resequencing, five years after initial negative ES results. The rate of physician-initiated routine re-evaluation was also monitored as part of the study. Of the 103 individuals included, physicians requested reevaluation for 45, which led to a total of 18 diagnoses (diagnostic yield of 31%). The study's systematic reevaluation then identified another 14 diagnoses (total diagnostic yield 53%). The new diagnoses were uncovered through the use of better bioinformatic pipelines, improved coverage after resequencing, reclassification of previously identified variants and new gene-disease associations. Notably, 11 of the 14 genetic diagnoses found via the systematic reevaluation were in children who did not recontact the referring physician. The authors conclude that both resequencing strategies as well as reanalysis of existing ES data are valuable in identifying additional genetic diagnoses. The study showed that not all afflicted individuals will undergo routine reevaluation, prolonging their diagnostic odyssey, unless a systematic reanalysis of negative results becomes standard.

Tan et al. (2020) performed an evaluation of the systematic reanalysis of ES for undiagnosed individuals and a literature review of studies that examined the reanalysis of ES data for cases in which a diagnosis was not found on initial ES. Data from 58 undiagnosed individuals was analyzed at 4-13 months post initial results, including evaluation of genes that had been newly linked with disease since the first analysis. A second reanalysis was performed 9-18 months after initial testing and considered all disease-related genes. Finally, at 25-34 months, all cases were reviewed with a comparison performed of the strategies used to identify a diagnosis. The study found that reanalysis of the existing ES data only (at two points in time) did not yield any new diagnoses, however the use of additional strategies such as repeat sequencing, trio sequencing and microarray detection of copy number variation led to 10 new diagnoses (17%) in this cohort. The literature review identified 27 peer-reviewed articles; median rate of new diagnosis subsequent to reanalysis was 15% and median time to reanalysis was



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22 months. Based on their study and review, the researchers suggest an interval of at least 18 months from the time of initial ES may be optimal, using diverse strategies for individuals who remain undiagnosed after individual ES.

To evaluate the ability of exome reanalysis to lead to a diagnosis, Wenger et al. (2017) performed reanalysis of exome and phenotypic clinical information from 40 individuals who had previously undergone WES with nondiagnostic results using up-to-date software and literature. The majority (28/40) had a neurologic or neurodevelopmental condition. For 10% of the participants, reanalysis led to a definitive diagnosis. At the time of their initial ES, literature linking causative genes to the phenotypes of the individuals studied was weak, nonexistent or difficult to locate. This is because approximately 250 gene-disease and 9,200 variant-disease associations are described yearly; per the authors, this necessitates regular reevaluation of previously nondiagnostic exomes. This study suggests reanalysis at a frequency of 2-3 year intervals could result in a 10% diagnostic yield. Larger studies are recommended to define standard timeframes for reanalysis with consideration for the evolving rate of discovery of relationships between genes and phenotypes and associated cost.

## **Whole Genome Sequencing (WGS)**

### **WGS: WGS-Pediatric (Non-Cancer)**

Currently, multiple different approaches may be used to genetically evaluate individuals with intellectual disability (ID) or neurodevelopmental disorders (NDDs). In a retrospective analysis including individuals who had been referred for diagnostic genetic testing at Karolinska University Hospital in Stockholm Sweden, Lindstrand et al. (2022) examined the results of testing from three different diagnostic methods in individuals with ID/NDD. In cohort 1, genome sequencing (GS) was used for first-line genetic evaluation (n=100). GS was used as second or third-line genetic testing (most commonly after CMA/FMR1 testing) when first-line testing was unsuccessful in identifying a cause for the clinical phenotype in cohort 2 (n=129). Finally, CMA (and FMR1 expansion testing in 50% of this group) was used in 421 participants (cohort 3). Noted commonalities across the groups were epilepsy and dysmorphic features. For cohort 1, using GS as a first-line test, diagnostic yield was 35%. When GS was used as a secondary test (cohort 2), yield was 26% and when only CMA/FMR1 was performed (cohort 3), yield was 11%. Of note, when GS was performed as a secondary test, age of diagnosis was delayed approximately one year and for those with a negative result after CMA/FMR1 testing (n=338), no referral for additional genetic testing was made (after 13 months) and individuals remained undiagnosed. The authors conclude that this study's findings support the use of genome evaluation over other testing strategies and should be used in place of CMA and FMR1 as a first-line test in individuals with DD/NDD.

In a prospective study evaluating children with global DD/ID, Sun et al. (2022) sought to assess the performance of GS for individuals whose CMA and ES results were inconclusive. One hundred children with global DD/ID who had received at least one genomic diagnostic test prior to enrollment were recruited for this study, which took place in China. The researchers reanalyzed CMA and ES results, calculating yield of GS and seeking explanations for diagnoses that were missed by CMA/ES. They found the overall diagnostic yield of GS to be 21% and determined that diagnoses could have been reached in seven cases with reanalysis of the ES data. Clinical utility was assessed via phone interview with parents; of the diagnosed families, nine experienced changes in clinical management which included adding targeted treatment, ending unnecessary treatment and consideration for family planning. The authors assert that use of GS led to high diagnostic yield and clinical utility for this study's participants.

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Xiao et al. (2022) performed a meta-analysis to summarize the utility of rapid GS in diagnosing critically ill infants. Twenty-three studies including 1567 infants met inclusion criteria and were analyzed. Overall, pooled diagnostic utility of rapid GS was 0.42 (95% CI: 0.37-0.49, I2 = 79%, P < 0.1). Taken together, diagnostic rates of rapid WES and WGS were 0.50 (95% CI: 0.41-0.61; I2 = 74%; P < 0.01 for WES) and 0.37 (95% CI: 0.30-0.46; I2 = 77%; P < 0.01) for WGS, indicating that rapid GS has good diagnostic utility in critically ill infants.

The Medical Genome Initiative is an association of leading research and health care groups in Canada and the United States focused on broadening access to WGS by bringing together experts in the field and publishing best practices. In 2022, this group published best practices for interpretation and reporting WGS (Austin-Tse et al., 2022). Key recommendations and unmet needs regarding requisitioning and consent, overall analysis, reporting and reanalysis were addressed. Clinical criteria for testing was not addressed in this document, but the authors do note that it is highly recommended that labs offer options for reanalysis of WGS cases.

In a Clinical Utility Evaluation (2022), Hayes identified no studies investigating the use of WGS in adults suspected to have neuromuscular or movement disorders when standard diagnostic testing was uninformative. Studies evaluating WGS data and its relationship to management and outcomes in individuals with these disorders are needed.

Kelly et al. (2022) used WGS to investigate blood pressures, first in a large group of multi-ancestry participants in the Trans-Omics for Precision Medicine and Centers for Common Disease Genomics program, and in a later stage, array data from UK Biobank (N=383,145), the Million Veteran Program (n=318,891) and Reasons for Geographic and Racial Differences in Stroke participants (n=10,643). This data was analyzed in tandem with WES data from UK Biobank (n=199,631). Genome-wide significance was found via meta-analysis in two blood pressure signals, one of which showed significance in both stages of the study. Nineteen additional signals were suggestively associated with blood pressure. The researchers concluded that a single promising, but unconfirmed rare variant was identified and recommend additional studies for the continuation of identification of rare variants associated with blood pressure.

Stranneheim et al. (2021) reported on the results of WGS for 4,437 individuals (3219 individuals and 1218 relatives) tested at the Genomic Medicine Center Karolinska-Rare Diseases (GMCK-RD) since mid-2015. Reporting included results from both individual (84%) and trio/family testing (16%). In total, 40% of individuals tested received a molecular diagnosis (ranging from 19% to 54% depending on specific disease groups). Common genes found to be causative included COL2A1 (skeletal dysplasia), SCN1A (epilepsy) and TNFRSF13B (inborn errors of immunity). Additionally, negative cases went on to be included in further studies, resulting in the identification of 17 new disease-causing genes. The use of WGS at GMCK-RD has resulted in diagnoses for over 1200 individuals with varying rare diseases. The authors advocate for continued clinical and academic partnership to expand the use of clinical WGS and help individuals with rare diseases end their diagnostic odysseys and gain understanding of their prognosis and treatment options.

Changes in medical care and changes in the cost of care were the primary outcome measures of a study evaluating the clinical and economic impact of the use of rapid whole genome sequencing (rWGS) as a first line diagnostic test for acutely ill infants (less than one year) old across five tertiary care children's hospitals in California (Dimmock et al. 2021). Project Baby Bear enrolled 184 critically ill babies, of whom 74 (40%) ultimately

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received diagnosis of a rare genetic disease after rWGS testing. Variants of uncertain significance (VUSs) were found in 21 babies (11%) and no diagnosis was obtained in 89 babies (48%). At least one change in medical care was implemented in cases of 58 babies. The authors concluded that overall, Project Baby Bear improved clinical outcomes in a real-world system through the use of rWGS. The project was not without limitations, however; physician- and parent-reported measures of harm and benefit were not systematically collected for evaluation and the findings may not be generalizable beyond the U.S. health care context.

A 2021 Hayes Clinical Utility Evaluation (2021a) indicates that evidence is insufficient to support the use of WGS to inform clinical action/improve outcomes in children 18 years or younger with neurological phenotypes who are lacking a diagnosis after standard diagnostic tests. Outcomes from available data are from a small and narrowly defined population group focused on infants with neurological phenotypes. Additional studies evaluating both larger numbers and a broader range of children with neurological symptoms are needed. An additional Clinical Utility Evaluation (Hayes, 2021b) found insufficient evidence for clinical utility related to the use of WGS in individuals with a primary phenotype of intellectual disability. This evaluation does not address intellectual disability in individuals with other disorders including neurodevelopmental disorders or global DD.

In a 2021 publication, Krantz et al. reported the results of their investigation of the effect of Whole Genome sequencing (WGS) on the impact of clinical management of infants admitted to an ICU from 5 US children's hospitals. Their multicenter randomized trial incorporated a time-delayed study design and focused on selection of children whose providers suspected genetic disorder. Usual care was continued through the study, capturing variation in management and helping with the assessment of real-world clinical situations. A total of 354 infants were enrolled from September 2017 to April 2019, with observation through July 2019. Infants between 0 and 120 days old were included (mean age = 15 days). The infants were randomized to receive WGS results either 15 days (early) or 60 days (delayed) after study enrollment. Infants were racially and ethnically diverse with a geographically distributed population in the US. The researchers indicated that twice as many infants in the early group vs the delayed group received a change in management (COM) (34 of 161 vs. 17 of 165) and molecular diagnosis (55 of 176 vs 27 of 178) at 60 days. COM and diagnostic efficacy doubled in the delayed group at 90 days (to 45 of 161 and 56/178, respectively). The study showed no measurable difference in length of stay or survival. The authors concluded that comprehensive genomic testing of acute care infants can impact clinical management and that WGS specifically positively impacts patient care and should be considered for critically ill infants with suspected genetic disease as a primary tool. Of note, this study was industry sponsored and conflicts of interest were present which could have impacted choice of methods (in particular, outcomes), or the validity of the interpretation of the findings. In addition, the findings may not be generalizable to ICUs outside of tertiary referral centers, which may have a lower incidence of genetic disease. The relevance of study findings on clinical outcomes is unclear and was not examined in this study.

In a 2021 preliminary report, Smedley et al. shared results of their pilot study investigating the role of genome sequencing in individuals with undiagnosed rare diseases. The study included 2,183 families with a total of 4,660 participants who were recruited after having been identified by health care providers and researchers as having rare diseases that had not yet been diagnosed after receipt of standard care (including no diagnostic testing or approved diagnostic tests which did not include genome

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sequencing) in the UK National Health Service. Among the participants, 161 disorders including a broad array of rare diseases, was present. Data was collected on clinical features, genome sequencing was performed, and new pathogenic variants were identified through the analysis. The disease categories of participants being evaluated for rare genetic conditions included: cardiovascular disorder, ciliopathy, dermatologic disorder, dysmorphic or congenital abnormality, endocrine disorder, gastroenterological disorder, growth disorder, hematologic or immunologic disorder, hearing or ear disorder, metabolic disorder, intellectual disability, neurologic or neurodevelopmental disorder, ophthalmologic disorder, renal and urinary tract disorder, respiratory disorder, rheumatologic disorder, skeletal disorder, or tumor syndrome. The report indicates that diagnostic yields were highest in families with larger pedigrees and were higher for disorders likely to have a monogenic cause (35%) than for disorders with a complex cause (11%). Fourteen percent of diagnoses were made using a combination of automated approaches and research which was especially important for cases with etiologic noncoding, structural and mitochondrial genome variants as well as variants which were not well covered by exome sequencing. In the course of the study, 3 new disease genes and 19 new associations were discovered. Ultimately, 25% of diagnoses that were made had immediate implications for clinical decision-making for affected individuals and their families. The researchers concluded that study showed an increase in diagnostic yield for rare diseases when genome sequencing was used and supports the case for using genomic sequencing when diagnosing certain specific rare diseases. However, the study did not include a comparison group and the relevance of the study findings on clinical outcome is only documented in the publication with anecdotal reports

Dimmock et al. (2020) reported the results of clinician surveys regarding the clinical utility of rapid whole genome sequencing (rWGS). Clinicians surveyed had cared for infants when genomic sequencing results were returned as part of the second Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT2) study. NSIGHT2 was a randomized controlled trial of rapid whole genome sequencing (rWGS), rapid whole exome sequencing (rWES) and ultra-rapid whole genome sequencing ([urWGS]; used for gravely ill infants) performed on infants with diseases of unknown etiology in intensive care units (ICUs). The goal of the NSIGHT study was to compare two methods of rapid genomic sequencing (rWGS or rWES) and two interpretation methods in acutely ill infants in terms of outcomes and utility. The clinician surveys used in this study found that clinicians perceived diagnostic genomic sequencing to either be useful or very useful for 77% of infants tested. Clinical management was reported to have been changed for 28% of infants, with greatest impact seen in those who received urWGS and positive test results. Rapid genomic sequencing was perceived to have changed outcomes for 15% of infants in the study. Clinicians did not perceive significant differences between WES vs WGS or between rapid or ultra-rapid sequencing in terms of clinical utility. Study results led the authors to conclude that broad use of genomic sequencing as a first-tier test for infants with diseases of unknown etiology in ICUs is associated with utility in over 75% of cases, management changes in more than 25% and outcome changes in 15% of infants. In addition, there was perceived communication improvement with 40% of families. The researchers feel that this data supports standard use of genomic sequencing for use in infants in ICUs. However, the clinicians' survey was not collected using a validated tool and the relevance of the study findings on clinical outcome is unclear and was not examined as part of this study.

Wang et al. (2020) used optimized trio genome sequencing (OTGS) to evaluate 130 pediatric patients who had clinical phenotypes suggestive of a genetic disorder from a pediatric intensive care unit (PICU)/neonatal intensive care unit (NICU) Chinese children's hospital. OTGS found pathogenic variants in 62/130 children resulting in a diagnostic

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yield of 47.7%. Of these children, 77% had single nucleotide variants, 19.4% had copy number variants and 3.2% had small deletions on one allele and another variant on an autosomal recessive gene. Of the children diagnosed, 48.4% had changes made to their clinical management strategies resulting in improved prognosis. The authors indicated that OTGS has the potential to be the first tier of genetic testing used in critically ill infants in developing countries, but further studies are needed.

A large study of WGS was performed by Turro et al. (2020) with patients in individuals who had rare diseases. The researchers aimed to use WGS in 83 national health systems and hospitals (UK and other countries) and had 13,037 participants. The participants ranged in age (from birth to 95 years of age), race, gender, and disorders. Of all participants these patients, 9,802 had a rare disease and 9,024 were probands; and 778 were affected relatives. A genetic diagnosis was defined for 1,138 of the 7,065 participants that were extensively phenotyped. The study identified 95 Mendelian associations between genes and rare diseases.

While following the American College of Medical Genetics (ACMG) guidelines to assess variant pathogenicity, Hou et al. (2020) conducted a prospective cohort study combining deep phenotyping with whole genome sequencing (WGS). Patients rticipants were adults (n = 1,190) who consented to WGS and agreed to participate in metabolomics, clinical laboratory testing, advanced imaging and provide family/medical history. Phenotypic results were, subsequently, integrated with genomic results. Positive pathogenic findings suggesting a genetic risk predisposition, were found in 17.3% of adults. When genetic results were incorporated with deep phenotyping, 11% had observed genotype/phenotype correlations. Greater than 75% of these correlations included risk for dyslipidemia (n = 24), cardiomyopathy, arrhythmia/other cardiac conditions (n = 42) and endocrine/diabetic conditions (n = 17). Approximately 6% of patientparticipants with pathogenic variants did not have a genotype/phenotype correlation. Hou et al. concluded that results of this study and future studies can provide beneficial information to aid in precision medical practice. The authors indicated that this study did not measure health outcomes or benefits. Repeat evaluation of these individuals is required to characterize the clinical significance of the findings.

NSIGHT2, a prospective, randomized, controlled and blinded trial of the clinical utility of rapid whole exome sequencing (rWES) and rapid whole genome sequencing (rWGS) on 1,248 critically ill infants from Rady Children's hospital, was performed by Kingsmore et al. (2019). Forty-six percent had conditions of unknown etiology and parent/child trio samples were available from 69% of participating families. Within 96 hours of hospital admission, 213/1,248 (37%) infants were enrolled and due to disease severity. Eleven% (24) received ultra-rapid whole genome sequencing (urWGS) and were not randomized. Of the remaining 189 infants, 95 were randomized to rWES and 94 to rWGS. The analytical performance of rWGS surpassed rWES including variants ClinVar pathogenic variants (p = 0.0001). The diagnostic performance was similar for rWGS and rWES yielding 19% vs 20%, respectively. Resulting time for diagnosis was also not significantly different 11 vs 11.2 days, respectively, for rWGS and rWES. The proportion of diagnosis made by urWGS (46%) was greater than that of rWES/rWGS (p = 0.004; result time was also less, p < 0.0001). Performing reflex trio testing following a negative proband result increased the diagnostic yield by 0.7%. Published data from NSIGHT2 yielded 92% clinical utility for the 24 patients-individuals undergoing urWGS and 73% clinical utility overall for the 189 infants who were randomized to rWGS and rWES. The authors concluded that rapid genome sequencing can be considered as a first-tier diagnostic test for inpatient, critically ill children. urWGS results in the shortest turnaround time which was crucial for those infants whose diagnosis will impact immediate medical management. The authors indicated



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that a direct comparison of the diagnostic performance of urWGS and rWES is warranted, with larger sample size than what was used for this study, and, ideally, performance of both tests in each proband.

Sanford et al. (2019) performed a retrospective cohort study evaluating the clinical utility of rapid whole genome sequencing (rWGS) in critically ill children. A single tertiary children's hospital pediatric intensive care unit (PICU) enrolled 38 children 4 months to 18 years with undiagnosed disease. rWGS was performed with targeted phenotype-driven analysis for patients and their parents when possible. A genetic diagnosis using rWGS was obtained in 17 (45%) of the patients. Pathogenic variants identified were associated with epilepsy, autoimmune, immunologic/inflammatory disorders and cardiomyopathy including ventricular dysrhythmia. A diagnostic yield of 30-50% was attained by rWGS in addition to a substantial time savings. Of the 17 patients with a genetic diagnosis, 4 had a change in medical management including genome-informed changes in medications. The researchers also stated that 82% of these diagnoses affected the clinical management of the patient after discharge. Additionally, 9 of the 17 diagnosed patients (53%) had no developmental delay or dysmorphic features. Sanford et al. concluded that data was limited in older children, but their report supports the findings of a previous study by Mestek-Boukhibar et al. (2018) that achieved a genetic diagnosis in 42% of 24 pediatric and cardiac ICU critically ill children. According to the authors, further studies are needed to identify PICU patients who will benefit from rapid whole genome sequencing early in PICU admission when the underlying etiology is unclear.

Children admitted to the pediatric intensive care unit are typically critically ill and require rapid clinical response. To determine if first tier WGS could impact patient outcomes, French et al. (2019) explored the clinical utility of trio WGS in 195 families who had a child in the PICU or NICU suspected of having a genetic disease or were referred to the study because of a suspected genetic diagnosis. A total of 567 samples were analyzed between the probands and family members, and a definitive diagnosis was found in 21% of cases. The average turn-around time from blood sample to report was three weeks. The phenotypic description of the child was not well aligned with the final genetic diagnosis in many cases, suggesting that phenotype should not drive the use of WGS in evaluating children in the PICU or NICU. In those receiving a genetic diagnosis, clinical management was changed in 65% of cases, and for those in the NICU cohort, management changed in 83%.

~~Scocchia et al. (2019) studied the use of WGS as a first-tier test at a resource limited dysmorphology clinic in northern Mexico as part of a collaboration between the Illumina iHope Program and the Foundation for the Children of the Californias and Hospital Infantil de Las Californias. Sixty children who were being followed as suspected of having a genetic diagnosis that was unresolved after expert clinical examination were tested with WGS. Ordering clinicians completed a survey that queried their change in management resulting from WGS results. Clinically relevant results were found in 68.3% (41) of patients. Nearly half were copy number variants or chromosomal abnormalities (20), including a mosaic trisomy. WGS results produced a new clinical diagnosis in 33 cases and confirmed a clinical diagnosis in eight. Clinicians reported a change in clinical management in 20 (48%) of cases, which included referrals to specialists not previously considered, for imaging, or functional testing. Muscle biopsy was avoided in three cases, and one patient was transferred to palliative care after a diagnosis of neuronal ceroid lipofuscinosis. In cases where no molecular diagnosis was found, clinicians reported that this information was helpful to the care of six patients by narrowing down the differential diagnosis, or expediting additional clinical workup, such as a muscle biopsy. One patient was enrolled in a clinical trial based on the~~



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~~identification of an interesting VUS. Additional studies are needed, but this suggests that WGS may be beneficial as a first-tier analysis in children suspected of having a genetic diagnosis.~~

Clark et al. (2019) described the analytical validity and clinical validity of an approach to rapid whole genome sequencing utilizing a platform designed for rapid, population scale sequencing using automated phenotyping and interpretation tools to make a provisional diagnosis. Conventional rapid WGS (rWGS) relies on preparing purified DNA from blood, DNA quality review, normalization of DNA concentration, preparation of the sequencing library, and library quality assessment. This platform instead relies on manually preparing libraries directly from blood samples or dried blood spots using microbeads with appropriate chromosomal segments (transposons). This method proved to be faster and less labor intensive. In four timed runs, the mean time to prepare the library was two hours and 45 minutes, as compared to ten hours by conventional methods. In the conventional approach, after preparation, samples were sequenced with the HiSeq 2500 sequencer in rapid run mode, with one sample processed per instrument, taking an average of 25 hours. In the modified approach, rWGS was performed on the NovaSeq6000 and S1 flow cell, as this instrument is faster with automated washing after a run. In four timed trials, sequencing took a mean of 15 hours and 32 minutes and yielded 404-537 Gb per flow cell, enough for two or three 40x genome sequences. Analysis of the sequence data was performed utilizing Dynamic Read Analysis for GENomics (DRAGEN), software that was optimized for speed, sensitivity and accuracy. Alignment and variant calling took a median of 1 hour and is similar to standard methods. Structural variants were not included. Analysis of relevant variants is typically achieved through filtering based on patient phenotype, and typically this is done by manual input of the patient's clinical features. Which features to select can be subjective and biased, and often incomplete. The team developed a natural language processing algorithm to extract clinical features from unstructured text in the EHR and optimized the algorithm from the training set used by Rady Hospital on 16 children with genomic disease and enriched with text used to identify children with orphan diseases. This included mapping 60% of Human Phenotype Ontology (HPO) terms and 75.4% of Orphanet Rare Disease HPO terms to SNOMED CT by lexical and logical methods and then manually verifying them. This set was then tested on a group of 10 children who had genome sequencing for genetic disease diagnosis to determine if the automated phenotype extraction from the EHR was reliable. A detailed manual review of the EHR was compared to the output of the algorithm, and the sensitivity was found to be 80%. To determine the clinical validity of this approach, the algorithm was compared in 101 children who had WGS where the phenotype to use for analysis was selected by a clinical expert. The algorithm identified 27-fold more phenotypic features than the expert manual selection, and four-fold more than if Online Mendelian Inheritance of Man (OMIM) terms alone were used. The process described was tested retrospectively in 95 children who had already had prior manual expert interpretation, and a second manual expert interpretation and the automated process were compared. The new manual expert interpretation was concordant with the prior results in 93 children, with two children being issued new reports with new revised diagnoses. The automated approach was concordant with the new manual review in 99% of cases, and with the prior manual review in 97% of cases. This process was tested prospectively in seven seriously ill infants in the NICU. The median time from blood sample to diagnosis for 19 hours and 56 minutes, compared with the standard testing time of 48 hours and 23 minutes. Three patients received a genetic diagnosis, confirmed by the standard method and Sanger sequencing. One patient's diagnosis was 16 hours earlier and another 27 hours earlier than the conventional approach resulting in earlier and more confident treatments than would have otherwise been considered.

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In order to analyze the application of WES and WGS as a routine diagnostic tool for patients, Smith et al. (2019) undertook a scoping review of the literature, following the Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) method of reporting observational studies. The timeframe from which they drew from the literature was 2009 to 2017, and they focused on diagnostic WES or WGS for infant and pediatric patients. A total of 171 articles were found, of which 131 were case reports, 40 were aggregate analysis and 4 were focused on a cost-effectiveness objective. The only metric consistently reported across all studies was diagnostic yield, and that varied broadly by clinical category and test type. In aggregate it was 33.2%. The authors concluded that multi-disciplinary research that focuses on consistency in outcome measurement is needed to demonstrate clinical utility.

~~Genetic hearing loss is heterogeneous and often difficult to diagnose. Sheppard et al. (2018) examined the clinical utility of WES on a cohort of 43 probands with hearing loss and compared the diagnostic yield with a common hearing loss genetic tests in a sub-group of 32 patients. WES focused the analysis on 247 genes associated with hearing loss, and targeted sequencing was up to the ordering clinician. Four patients had a four gene panel, 19 had a 70 gene panel, and five had single gene testing. Twenty-four had a chromosome microarray performed, and a number of patients had more than one targeted test. Overall, the diagnostic yield was 37.2% for WES, compared to 15.8% of the common hearing loss genetic tests.~~

The clinical utility of rapid WGS in the pediatric intensive care unit (PICU) was the subject of a study by Mestek-Boukhibar et al. (2018). They set up a multi-disciplinary team to identify patients at a tertiary NHS tertiary children's hospital with a 23-bed multidisciplinary PICU and a 20-bed pediatric cardiac intensive care unit (CICU). Patients were considered for inclusion that were considered critically ill, but death was not imminent, had parents available for testing, and had a suspected heterogeneous monogenic disease with possible treatment available. An initial ten trios were used as a training set for the rapid sequencing and bioinformatics approach from which the team learned and revised the process for further optimization. A final 14 trios went through the finalized process. Overall, a primary molecular diagnosis was found in ten of 24 trios (42%). The shortest time to complete the full workflow, which included patient identification, informed consent, sample collection, and testing, was 5 days. There was impact on clinical management was significant for three patients. One child had a ruptured spleen and child protection concerns had been raised, but WGS identified Ehler-Danlos syndrome which explained the phenotype. Another child with renal failure had a pathogenic variant in the *WT1* gene, resulting in bilateral nephrectomy to prevent the development of Wilms tumors. The third child was diagnosed with Sotos syndrome and had an atypical presentation that in other situations may not have been diagnosed for some time. The early diagnosis allowed for early endocrine management of the disease. In addition, one child who did not have a primary molecular diagnosis was found to have a homozygous *BCHE* mutation that conveys a risk of apnea with certain anesthesia. This altered the medical management as the child had to undergo several surgical procedures. The authors noted that traditional, targeted testing for these individuals may not have been done due to atypical presentations, or the results could have taken as long as 8 weeks. It was highlighted that this process required close and rapid communication with the clinical team, lab, and computational systems, and required optimization and revision of standard processes to meet the aggressive timeline.

Clark et al. (2018) conducted a meta-analysis comparing the diagnostic and clinical utility of WGS, WES and chromosome microarray (CMA) in children suspected of having genetic disease. Analysis of the literature from January 2011 to August 2017 was

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conducted following the Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) and Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines. Thirty-seven studies of 20,068 children were included. Overall, the diagnostic utility of WES and WGS was greater than CMA. In studies from only 2017, the diagnostic utility of WGS was greater than CMA. Among studies featuring in cohort comparisons, the diagnostic utility of WES was greater than CMA. The diagnostic utility between WGS and WES was not significantly different. In studies with in-cohort comparisons of WGS and WES, there was a greater chance of achieving a diagnosis when a trio was available than singleton testing, and with in-hospital interpretation versus a reference lab interpretation. In this study, clinical utility was defined as a change in clinical management. Cases where the only change was reproductive planning, or a change in genetic counseling, were excluded. The clinical utility of WES was greater, but not statistically significant, than CMA. However, WGS was higher for clinical utility than CMA, and met statistical significance ( $p < 0.0001$ ). The authors identified several limitations with the meta-analysis, such as the heterogeneity of the pooled data, taking diagnostic rates at face value, and that only one study met the highest level of evidence criteria for clinical interventions. Overall, they concluded that more randomized, well designed and controlled clinical studies are needed but WES and WGS could be considered over CMA for a first-tier test in a child suspected of having a genetic diagnosis.

Petrikin et al. (2018) conducted a partially blinded randomized control trial on the clinical utility of rapid whole genome sequencing (rWGS) in neonatal intensive care unit/pediatric intensive care unit patients from October 2014 to June 2016. Eligible patients were < 4 months old and had illnesses suggestive of a genetic disease, but were of unknown etiology. The studied intervention was trio rWGS, meaning WGS testing was completed in about 2 days, and was performed on the infant and parents. rWGS results were confirmed by another testing method prior to clinical reporting unless a situation arose where life-threatening progression was likely. There were 129 infants in the study period that were potential candidates, and 65 (50%) were ultimately enrolled. Thirty-two infants were randomized to rWGS plus standard genetic testing, and the remaining 33 had standard genetic testing alone. Standard genetic testing was defined as any genetic test considered standard of care, and therefore available to order through the electronic medical record. During the study period, non-rapid WGS became available, and was considered a standard test. The baseline characteristics of the infants in both groups were similar. The most common indications were congenital anomalies and neurological disorders. In the control group, only 6% of the infants had cardiovascular findings compared to the rWGS group (28%), and this may have impacted the likelihood of genetic disease. Other than newborn screening, the average age at first test order was 14 days. In those that had standard genetic testing, a diagnosis was identified by the test in 23% (7) of test cases, and 24% (8) of controls. The diagnostic rate by type of test included 6% by chromosome microarray, 18% by targeted panel testing, 33% by WES, and 13% by methylation testing. In this group, it is noted that rWGS would not identify 33%, or five of fifteen diagnoses, as four were structural variants and one was a change in DNA methylation not identifiable at the time of this study by rWGS. The median time from test order to diagnosis was 64 days. In the test group, rWGS identified a diagnosis in 31% (10) cases, with a median time to diagnosis of 14 days, which included confirmatory testing. After un-blinding the randomization after 10 days of enrollment, it was requested by participating physicians to allow 7 of the 33 controls to participate in rWGS. It was declined for two patients as they were not acutely ill and about to be discharged. The remaining 5 had rWGS, and 2 received a diagnosis by rWGS that was later confirmed by standard genetic testing that was already being performed. Overall, infants receiving trio rWGS had a higher rate of diagnosis and shorter time to diagnosis than infants receiving standard tests alone. The ability of this study to understand the

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clinical utility of rWGS was hampered by the cross-over requests after 10 days of enrollment un-blinding, and the increasing availability of targeted panel tests, WES and WGS during the study period as standard tests, which ultimately caused the study to end early. The authors concluded that rWGS trended toward earlier diagnosis in the NICU, prior to discharge, but more studies are needed to determine if a shorter time to diagnosis improves clinical utility, outcomes, or healthcare utilization.

In another view of rWGS in acutely ill infants, Farnaes et al. (2018) provides a retrospective review of inpatients from July 2016 to March 2017. A total of 42 families received rWGS plus standard of care genetic testing for the purpose of diagnosing genetic disorders. Trio testing was performed on 29 cases, 1 quad (parents plus two affected children), 9 duos (mother infant) and three infants only. The majority of infants in the study were Hispanic/Latino (59%), and were in a neonatal intensive care unit (NICU), pediatric intensive care unit (PICU) or cardiovascular care unit (71%), on respiratory support (76%), and inotropic support (40%). The most common clinical indication was multiple congenital anomalies (29%). There was little consanguinity (2%). In examining the standard genetic testing results, the authors note that 4 infants received a diagnosis from these results. The most common standard test was chromosome microarray, but routine chromosome analysis, fluorescent in situ hybridization, and various biochemical tests were also utilized. One infant had a change in care as a result of the diagnosis. rWGS provided a diagnosis in 18 infants. All findings were confirmed through standard genetic tests. Thirteen children had a change in care as a result which included starting new medications (5), discontinuing medications (2), surgical procedures were changed (4). Palliative care was planned for one infant. Overall, the authors concluded that the availability of the rWGS results allowed changes in care that prevented morbidities in 11 of the infants and significant risk reduction in acute mortality in 1 from a medication change. In summary, the authors found that the diagnostic sensitivity in this cohort was 43% for rWGS and 10% for standard genetic testing, and concluded that rWGS may benefit acutely ill inpatient infants as a first tier test, but further studies are needed.

Bowling et al. (2017) report results of WES or WGS on 371 individuals with developmental delay or intellectual disabilities enrolled in the Clinical Sequencing Exploratory Research (CSER) consortium (WES for 127 and WGS for 244). A total of 284 participating families were enrolled with both biological parents and 35 affected individuals had one parent included. Mean age of study participants was 11 years and 58% were male. Affected individuals displayed symptoms described by 333 unique Human Phenotype Ontology terms with over 90% of individuals displaying intellectual disability, 69% with speech delay, 45% with seizures, and 20% with microcephaly or macrocephaly; 18% had an abnormal brain magnetic resonance imaging (MRI) result and 81% had been subjected to prior genetic testing. Pathogenic or likely pathogenic variants were found in 100 individuals (27%), with variants of uncertain significance in an additional 42 (11%). The pathogenic or likely pathogenic identification rate was not significantly different between WES or WGS ( $p=0.30$ ) for single nucleotide variants or small insertions or deletions, although WGS can also identify copy number variants.

In a prospective study, Stavropoulos et al. (2016) utilized WGS and comprehensive medical annotation (CMA) to assess 100 patients referred to a pediatric genetics service, and compared the diagnostic yield versus standard genetic testing. WGS identified genetic variants meeting clinical diagnostic criteria in 34% of cases, representing a fourfold increase in diagnostic rate over CMA alone and more than twofold increase in CMA plus targeted gene sequencing. WGS identified all rare clinically significant CNVs that were detected by CMA. In 26 patients, WGS revealed indel and missense mutations presenting in

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~~a dominant (63%) or a recessive (37%) manner. The investigators found four subjects with mutations in at least two genes associated with distinct genetic disorders, including two cases harboring a pathogenic CNV and SNV. In the authors' opinion, when considering medically actionable secondary findings in addition to primary WGS findings, 38% of patients would benefit from genetic counselling. While promising, additional studies of WGS as a primary test in comparison to conventional genetic testing and WES are needed.~~Splinter et al. (2018) reported on the findings of the Undiagnosed Diseases Network (UDN) which reported a diagnostic yield of 13% for whole genome sequencing (WGS) in persons that had undergone prior genetic testing, including WES, with no diagnosis. Patients (n = 601) that were accepted by the UDN were evaluated by WGS (192 had previously had WES). The majority of clinical phenotypes included 40% neurological, 10% musculoskeletal, 7% immunologic, 7% gastrointestinal and 6% rheumatologic. Complete evaluation was performed in 382/601, and WGS provided a result in 132 patients (35% diagnostic yield). Eleven percent (15 cases) of diagnoses were made solely by clinical review; 11% were made by directed clinical testing; 4% were made by non-sequencing genetic testing; 74% were made by WGS. Twenty-eight percent (55/195) of patients, who had WES performed, received a diagnosis; 32/165 (19%) of patients having WGS revealed a diagnosis. Seventeen of these patients (53%) had previously undergone unsuccessful WES testing prior to referral to UDN. Thirty-one new syndromes were revealed. Twenty-one percent of the diagnosis resulted in recommendations in therapy changes, 37% resulted in changes to diagnostic testing and 36% led to genetic counseling for variant discussion.

~~Bodian et al. (2016) assessed the potential of WGS to replicate and augment results from conventional blood-based newborn screening (NBS). Research-generated WGS data from an ancestrally diverse cohort of 1,696 infants and both parents of each infant were analyzed for variants in 163 genes involved in disorders included or under discussion for inclusion in US NBS programs. WGS results were compared with results from state NBS and related follow-up testing. NBS genes are generally well covered by WGS. There is a median of one (range: 0-6) database-annotated pathogenic variant in the NBS genes per infant. Results of WGS and NBS in detecting 28 state-screened disorders and four hemoglobin traits were concordant for 88.6% of true positives (n = 35) and 98.9% of true negatives (n = 45,757). Of the five infants affected with a state-screened disorder, WGS identified two whereas NBS detected four. WGS yielded fewer false positives than NBS (0.037 vs. 0.17%) but more results of uncertain significance (0.90 vs. 0.013%). The authors concluded that WGS may help rule in and rule out NBS disorders, pinpoint molecular diagnoses, and detect conditions not amenable to current NBS assays. There is a need for additional studies that compare WGS with traditional NBS methods and evaluate the change in patient management resulting from WGS for newborn screening.~~

~~Taylor et al. (2015) conducted a study to assess factors influencing the success of WGS to obtain a genetic diagnosis across a broad range of clinical conditions with no previously identified causal mutation. They sequenced 217 individuals from 156 independent cases or families across a broad spectrum of disorders in which previous screening had identified no pathogenic variants. The investigators quantified the number of candidate variants identified using different strategies for variant calling, filtering, annotation and prioritization. They 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, the investigators 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. They also discovered 32 potentially clinically actionable~~

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~~variants in 18 genes unrelated to the referral disorder, although only 4 were ultimately considered reportable. According to the investigators, their results demonstrate the value of genome sequencing for but also highlight many outstanding challenges, including the challenges of interpreting unrelated variants.~~

~~Willig et al. (2015) performed a retrospective comparison of rapid whole-genome sequencing (STATseq) and standard genetic testing in a case series from the neonatal and pediatric intensive care units (NICU and PICU) of a large children's hospital. The participants were families with an infant younger than 4 months with an acute illness of suspected genetic cause. The intervention was STATseq of trios (both parents and their affected infant). The main measures were the diagnostic rate, time to diagnosis, and rate of change in management after standard genetic testing and STATseq. Twenty (57%) of 35 infants were diagnosed with a genetic disease by use of STATseq and three (9%) of 32 by use of standard genetic testing. Median time to genome analysis was 5 days (range 3-153) and median time to STATseq report was 23 days. Thirteen (65%) of 20 STATseq diagnoses were associated with de novo mutations. Impact on clinical management was noted in 13 (65%) of 20 infants with a STATseq diagnosis, four (20%) had diagnoses that led to a clinical intervention and six (30%) were started on palliative care. The 120-day mortality was 57% (12 of 21) in infants with a genetic diagnosis. According to the authors, in selected acutely ill infants, STATseq had a high rate of diagnosis of genetic disorders. The authors indicated that while having a genetic diagnosis altered the management of infants in the NICU or PICU in this single institution, additional studies with a higher patient population are needed to validate the clinical utility of WGS in this patient population.~~

~~Soden et al. (2014) reported on one hundred families with 119 children affected by neurodevelopmental disorders (NDD) who had WGS, WES, or WES followed by WGS of parent-child trios, with the sequencing approach guided by acuity of illness. Forty-five percent received molecular diagnoses. An accelerated sequencing modality, rapid WGS, yielded diagnoses in 73% of families with acutely ill children (11 of 15). Forty percent of families with children with nonacute NDD, followed in ambulatory care clinics (34 of 85), received diagnoses: 33 by WES and 1 by staged WES then WGS. A change in clinical care or impression of the pathophysiology was reported in 49% of newly diagnosed families. According to the authors, if WES or WGS had been performed at symptom onset, genomic diagnoses may have been made 77 months earlier. It is suggested that initial diagnostic evaluation of children with NDD should include trio WGS or WES, with extension of accelerated sequencing modalities to high-acuity patients. According to the authors, this study had several limitations. It was retrospective and lacked a control group. Clinical data were collected principally through chart review, which may have led to under- or overestimates of changes in clinical management. The authors did not ascertain information about long-term consequences of diagnosis, such as the impact of genetic counseling. Comparisons of costs of genomic and conventional diagnostic testing excluded associated costs of testing, such as outpatient visits, and may have included tests that would nevertheless have been performed, irrespective of diagnosis. The acuity-based approach to expedited WGS and non-expedited WES was a patient care-driven approach and was not designed to facilitate direct comparisons between the two methods.~~

### WGS-Other (Non-Cancer)

Alfares et al. (2018) examined the clinical utility of whole genome sequencing compared to re-analysis of whole exome sequencing. All cases that underwent CAP accredited CLIA lab WES and WGS in the genetics clinic of King Abdulaziz Medical City between 2013-2017 were examined, regardless of phenotype. WES was performed on either an Illumina NextSeq or HiSeq, or on an Ion Proton system. The average coverage depth was



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95X. WGS was performed on a HiSeq 4000. The average coverage depth was 30X. Variant call files (VCF) were obtained for each case, and raw data analysis was performed in cases where the final results showed discrepancies. Discrepancies were classified into three categories; due to the time interval between tests, new discoveries could explain the discrepancy, intronic or large copy number variants may not have been seen due to WES limitations, and finally, the type of sequencing system could have created the discrepancy. Overall, 154 patients were included in the study and had negative comparative genome array results with, ~~and~~ had negative or inconclusive WES results. Most were male (56%), pediatric (91%) and consanguineous (70%). Forty-six were eventually excluded because WGS results were incomplete, additional testing was required, or WES VCF were not available from prior testing. The remaining 108 patients had complete clinical information and final WES and WGS results available. Of these, 10 patients had positive WGS results with prior negative WES results, and 5 had inconclusive results. The remaining 93 had negative WGS results. The average time between WES testing and WGS testing was only 5 months, and in that time no new clinical information was collected on the 10 positive WGS patients. However, in 3 cases, variants were found in WES, but not reported, because the data that demonstrated their pathogenicity was published after the initial WES was completed. In addition, four cases that had WES performed by the Ion Proton system missed variants that were anticipated to be found by WES. Original raw data files were not available from this lab to determine if the variants were present but filtered out, or if the genes were not adequately covered. Additional WES analysis using the Illumina system in these patients detected these four variants. Overall, only 3 cases were positive by WGS that were completely unidentifiable by WES. The authors concluded that in the final 108 patients, if they had re-analyzed the original WES data, they would have identified 30% of the positive cases, and that WGS only achieved a 7% higher detection rate. It was concluded that for this population re-analysis of WES data before, or in lieu of WGS, may have better clinical utility. Limitations of this study include the small sample size and the high rate of consanguinity, which may have resulted in a disproportionate number of positives on the initial WES test, which could in general limit the utility of WGS in the study population.

Another study that reviewed the utility of WES and WGS was conducted by Carss et al. (2017). The authors studied a cohort of 722 individuals with inherited retinal disease (IRD) who had WES (n = 72), WGS (n = 605) or both (n = 45) as part of the NIHR-BioResource Rare Diseases research study. The diagnoses included in the cohort included retinitis pigmentosa (n = 311), retinal dystrophy (n = 101), cone-rod dystrophy (n = 53), Stargardt disease (n = 45), macular dystrophy (n = 37), and Usher syndrome (n = 37). In the 117 individuals who had WES, 59 (50%) had pathogenic variants identified. Forty-five individuals with a negative WES had subsequent WGS, and an additional 14 pathogenic variants were found. In three of these, the variant location was absent from the WES hybrid capture kit. Three individuals had large copy number variants that could not be called by WES, and three others had variants that were found in the WES results, but the quality was poor, and they were not called. In the remaining 5 individuals, the variants were also found in WES, but the mode of inheritance was unexpected, so WGS was used to exclude other possible causes of the disease. The detection rate varied by phenotype, ranging from 84% in individuals with Usher syndrome to 29% in those with cone dystrophy. Ethnicity also impacted the detection rate. Only 30% of individuals with African ancestry had cases solved, compared to 55% of European ancestry or 57% of South Asian ancestry. The authors further reviewed benefits of WGS. They noted that 3 individuals had pathogenic, non-coding variants that would not be detected by WES. They compared the IRD genes that were high or low in GC content in their WGS data set to the same genes in the WES ExAC database and concluded that the WGS dataset had consistent coverage whereas the WES data did not. They also noted that in their data set, WGS was better at detecting

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synonymous variants and variants in regulatory regions compared to WES. Overall, the detection rate for WGS was 56% in this cohort. Factors that may influence this study compared to others is the technology used, phenotype screening and phenotypes used. They observed that the subset of people tested who had no prescreening had a higher pathogenic call rate, suggesting that the cohort may have been enriched for difficult cases, and the detection rate for WGS could be higher if used as a first line test. The authors noted that their WES coverage rate was 43X, compared to the > 80X recommended for a commercial lab, and that might have influenced the results.

Additional peer-reviewed literature on WGS consists primarily of case reports and small case series (Willig et al., 2015; Yuen et al., 2015; Jiang et al., 2013). The limited clinical experience with WGS causes gaps in interpreting variants of uncertain significance or other incidental findings. As a result, the benefits and risks of WGS testing are poorly defined and the role of WGS in the clinical setting has not yet been established.

WGS: ~~Forty~~ Bowling et al. (2017) report results of WES or WGS on 371 individuals with developmental delay or intellectual disabilities enrolled in the Clinical Sequencing Exploratory Research (CSER) consortium (WES for 127 and WGS for 244) A total of 284 participating families were enrolled with both biological parents and 35 affected individuals had one parent included. Mean age of study participants was 11 years and 58% were male. Affected individuals displayed symptoms described by 333 unique Human Phenotype Ontology terms with over 90% of individuals displaying intellectual disability, 69% with speech delay, 45% with seizures, and 20% with microcephaly or macrocephaly; 18% had an abnormal brain magnetic resonance imaging (MRI) result and 81% had been subjected to prior genetic testing. Pathogenic or likely pathogenic variants were found in 100 individuals (27%), with variants of uncertain significance in an additional 42 (11%). The pathogenic or likely pathogenic identification rate was not significantly different between WES or WGS ( $p = 0.30$ ) for single nucleotide variants or small insertions or deletions, although WGS can also identify copy number variants.

In a prospective study, Stavropoulos et al. (2016) utilized WGS and comprehensive medical annotation (CMA) to assess 100 patients referred to a pediatric genetics service and compared the diagnostic yield versus standard genetic testing. WGS identified genetic variants meeting clinical diagnostic criteria in 34% of cases, representing a fourfold increase in diagnostic rate over CMA alone and more than twofold increase in CMA plus targeted gene sequencing. WGS identified all rare clinically significant CNVs that were detected by CMA. In 26 patients, WGS revealed indel and missense mutations presenting in a dominant (63%) or a recessive (37%) manner. The investigators found four subjects with mutations in at least two genes associated with distinct genetic disorders, including two cases harboring a pathogenic CNV and SNV. In the authors' opinion, when considering medically actionable secondary findings in addition to primary WGS findings, 38% of patients would benefit from genetic counselling. While promising, additional studies of WGS as a primary test in comparison to conventional genetic testing and WES are needed.

A significant limitation of WGS is that the functional implications of variants outside the exons are relatively unknown (Klein and Foroud, 2017). To date, only a small number of research articles have addressed the clinical utility of WGS. Recently, several small studies have addressed the analytical validity of WGS as

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compared to WES and found that WGS may provide more uniform coverage than WES, may more accurately detect a small number of variants compared to WES, and be better at identifying copy number variants (Belkadi et al., 2015; Meienberg et al., 2016). However, due to the data complexity, processing time and interpretation time is much greater for WGS than for other NGS approaches (Klein and Foroud, 2017).

### WGS-Other (Non-Cancer)

~~In a 2021 preliminary report, Smedley et al. shared results of their pilot study investigating the role of genome sequencing in individuals with undiagnosed rare diseases. The study included 2183 families with a total of 4660 participants who were recruited after having been identified by health care providers and researchers as having rare diseases that had not yet been diagnosed after receipt of standard care (including no diagnostic testing or approved diagnostic tests which did not include genome sequencing) in the UK National Health Service. Among the participants, 161 disorders including a broad array of rare diseases, was present. Data was collected on clinical features, genome sequencing was performed, and new pathogenic variants were identified through the analysis. The report indicates that diagnostic yields were highest in families with larger pedigrees and were higher for disorders likely to have a monogenic cause (35%) than for disorders with a complex cause (11%). Fourteen percent of diagnoses were made using a combination of automated approaches and research which was especially important for cases with etiologic noncoding, structural and mitochondrial genome variants as well as variants which were not well covered by exome sequencing. In the course of the study, 3 new disease genes and 19 new associations were discovered. Ultimately, 25% of diagnoses that were made had immediate implications for clinical decision-making for affected individuals and their families. The researchers concluded that study showed an increase in diagnostic yield for rare diseases when genome sequencing was used and supports the case for using genomic sequencing when diagnosing certain specific rare diseases. However, the study did not include a comparison group and the relevance of the study findings on clinical outcome is only documented in the publication with anecdotal reports.~~

~~A large study of WGS was performed by Turro et al. (2020) with patients who had rare diseases. The researchers aimed to use WGS in 83 national health systems and hospitals (UK and other countries) and had 13,037 participants. The participants ranged in age (from birth to 95 years of age), race, gender, and disorders. Of these patients, 9,802 had a rare disease and 9,024 were probands and 778 were affected relatives. A genetic diagnosis was defined for 1,138 of the 7,065 participants that were extensively phenotyped. The study identified 95 Mendelian associations between genes and rare diseases.~~

~~While following the American College of Medical Genetics (ACMG) guidelines to assess variant pathogenicity, Hou et al. (2020) conducted a prospective cohort study combining deep phenotyping with whole genome sequencing (WGS). Patients were adults (n = 1,190) who consented to WGS and agreed to participate in metabolomics, clinical laboratory testing, advanced imaging and provide family/medical history. Phenotypic results were, subsequently, integrated with genomic results. Positive pathogenic findings suggesting a genetic risk predisposition, were found in 17.3% of adults. When genetic results were incorporated with deep phenotyping, 11% had observed genotype/phenotype correlations. Greater than~~

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~~75% of these correlations included risk for dyslipidemia (n = 24), cardiomyopathy, arrhythmia/other cardiac conditions (n = 42) and endocrine/diabetic conditions (n = 17). Approximately 6% of patients with pathogenic variants did not have a genotype/phenotype correlation. Hou et al. concluded that results of this study and future studies can provide beneficial information to aid in precision medical practice. The authors indicated that this study did not measure health outcomes or benefits. Repeat evaluation of these individuals is required to characterize the clinical significance of the findings.~~

~~Splinter et al. (2018) reported on the findings of the Undiagnosed Diseases Network (UDN) which reported a diagnostic yield of 13% for whole genome sequencing (WGS) in persons that had undergone prior genetic testing, including WES, with no diagnosis. Patients (n = 601) that were accepted by the UDN were evaluated by WGS (192 had previously had WES). The majority of clinical phenotypes included 40% neurological, 10% musculoskeletal, 7% immunologic, 7% gastrointestinal and 6% rheumatologic. Complete evaluation was performed in 382/601, and WGS provided a result in 132 patients (35% diagnostic yield). Eleven percent (15 cases) of diagnoses were made solely by clinical review; 11% were made by directed clinical testing; 4% were made by non-sequencing genetic testing; 74% were made by WGS. Twenty-eight percent (55/195) of patients, who had WES performed, received a diagnosis; 32/165 (19%) of patients having WGS revealed a diagnosis. Seventeen of these patients (53%) had previously undergone unsuccessful WES testing prior to referral to UDN. Thirty-one new syndromes were revealed. Twenty-one percent of the diagnosis resulted in recommendations in therapy changes, 37% resulted in changes to diagnostic testing and 36% led to genetic counseling for variant discussion.~~

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~~to determine if the variants were present but filtered out, or if the genes were not adequately covered. Additional WES analysis using the Illumina system in these patients detected these four variants. Overall, only 3 cases were positive by WGS that were completely unidentifiable by WES. The authors concluded that in the final 108 patients, if they had re-analyzed the original WES data, they would have identified 30% of the positive cases, and that WGS only achieved a 7% higher detection rate. It was concluded that for this population re-analysis of WES data before, or in lieu of WGS, may have better clinical utility. Limitations of this study include the small sample size and the high rate of consanguinity, which may have resulted in a disproportionate number of positives on the initial WES test, which could in general limit the utility of WGS in the study population.~~

~~Another study that reviewed the utility of WES and WGS was conducted by Carss et al. (2017). The authors studied a cohort of 722 individuals with inherited retinal disease (IRD) who had WES (n = 72), WGS (n = 605) or both (n = 45) as part of the NIH BioResource Rare Diseases research study. The diagnoses included in the cohort included retinitis pigmentosa (n = 311), retinal dystrophy (n = 101), cone rod dystrophy (n = 53), Stargardt disease (n = 45), macular dystrophy (n = 37), and Usher syndrome (n = 37). In the 117 individuals who had WES, 59 (50%) had pathogenic variants identified. Forty-five individuals with a negative WES had subsequent WGS, and an additional 14 pathogenic variants were found. In three of these, the variant location was absent from the WES hybrid capture kit. Three individuals had large copy number variants that could not be called by WES, and three others had variants that were found in the WES results, but the quality was poor, and they were not called. In the remaining 5 individuals, the variants were also found in WES, but the mode of inheritance was unexpected, so WGS was used to exclude other possible causes of the disease. The detection rate varied by phenotype, ranging from 84% in individuals with Usher syndrome to 29% in those with cone dystrophy. Ethnicity also impacted the detection rate. Only 30% of individuals with African ancestry had cases solved, compared to 55% of European ancestry or 57% of South Asian ancestry. The authors further reviewed benefits of WGS. They noted that 3 individuals had pathogenic, non-coding variants that would not be detected by WES. They compared the IRD genes that were high or low in GC content in their WGS data set to the same genes in the WES ExAC database and concluded that the WGS dataset had consistent coverage whereas the WES data did not. They also noted that in their data set, WGS was better at detecting synonymous variants and variants in regulatory regions compared to WES. Overall, the detection rate for WGS was 56% in this cohort. Factors that may influence this study compare to others is the technology used, phenotype screening and phenotypes used. They observed that the subset of people tested who had no prescreening had a higher pathogenic call rate, suggesting that the cohort may have been enriched for difficult cases, and the detection rate for WGS could be higher if used as a first line test. The authors noted that their WES coverage rate was 43X, compared to the > 80X recommended for a commercial lab, and that might have influenced the results.~~

~~Ellingford et al. (2016) compared the use of next generation gene targeted next generation sequencing (NGS) with WGS in a nested cohort of 46 (of 562) people with inherited retinal disease (IRD). Targeted sequencing and WGS were found to have a similar sensitivity and specificity, but WGS identified an additional 14 clinically relevant variants. If applied to the whole cohort of 562, the authors hypothesized~~



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~~that WGS would provide an overall 29% (95% confidence interval, 15-45) uplift in diagnostic yield. They also noted, however, that creating a more targeted NGS panel would have a similar result.~~

~~Cirino et al. (2014) examined the validity of WGS in 41 patients with hypertrophic cardiomyopathy (HCM) who had undergone a HCM targeted next generation sequencing panel test. Twenty of the participants had pathogenic variants identified by targeted sequencing, and WGS detected 19 of them. Three additional variants were found in genes associated with HCM, but these genes are not typically included in HCM targeted sequencing panels. Additionally, 84 secondary (incidental) findings were uncovered. The authors concluded that WGS may provide advantages in being able to interrogate more genes, and give the opportunity for re-analysis over time, but noted that expertise in genomic interpretation is required to incorporate into care.~~

~~Dewey et al. (2014) conducted a pilot study to determine the resources required to identify and interpret clinically relevant genetic variation using WGS technologies and to evaluate clinical action prompted by WGS findings. An exploratory study of WGS was conducted in 12 adult participants at Stanford University Medical Center between November 2011 and March 2012. A multidisciplinary team reviewed all potentially reportable genetic findings. Five physicians proposed initial clinical follow-up based on the genetic findings. Depending on sequencing platform, 10% to 19% of inherited disease genes were not covered to accepted standards for single nucleotide variant discovery. Genotype concordance was high for previously described single nucleotide genetic variants (99%-100%) but low for small insertion/deletion variants (53%-59%). Curation of 90 to 127 genetic variants in each participant required a median of 54 minutes per genetic variant, resulted in moderate classification agreement between professionals, and reclassified 69% of genetic variants cataloged as disease-causing in mutation databases to variants of uncertain or lesser significance. Two to 6 personal disease-risk findings were discovered in each participant, including 1 frameshift deletion in the BRCA1 gene implicated in hereditary breast and ovarian cancer. Physician review of sequencing findings prompted consideration of a median of 1 to 3 initial diagnostic tests and referrals per participant, with fair interrater agreement about the suitability of WGS findings for clinical follow-up. The authors concluded that in this exploratory study of 12 volunteer adults, the use of WGS was associated with incomplete coverage of inherited disease genes, low reproducibility of detection of genetic variation with the highest potential clinical effects, and uncertainty about clinically reportable findings.~~

~~Additional peer-reviewed literature on WGS consists primarily of case reports and small case series (Willig et al., 2015; Yuen et al., 2015; Jiang et al., 2013). The limited clinical experience with WGS causes gaps in~~



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~~interpreting variants of uncertain significance or other incidental findings. As a result, the benefits and risks of WGS testing are poorly defined and the role of WGS in the clinical setting has not yet been established.~~

## WGS-Cancer

One hundred and eighty-four consecutive patients with cancer were recruited into a study examining the clinical utility of WGS at the Oxford University Hospitals Foundation Trust's Genomics Medicine Center as part of the 100,000 Genomes Project. All patients were undergoing surgical resection, and tissue samples collected from adjacent region tissue blocks were prepared as both fresh frozen (FF) and formalin-fixed, paraffin-embedded (FFPE). Peripheral blood was obtained as a comparator as well. A significant number of samples were lost due to poor quality; 87 had a poor FF sample, and 30 had poor FFPE results. Another 15 patients were excluded at other quality control steps. In the end, a total of 52 cancer patients had a usable trio of sample sets that included ten breast, 12 colorectal, seven endometrial, four prostate, 14 renal, and five thoracic cancers. The quality and quantity of DNA between FF and FFPE was evaluated for these 52 patients, and it was noted that FFPE typically had short, denatured DNA, and resulting sequence data revealed non-uniform coverage. Different results were found between FF and FFPE samples in terms of somatic SNVs and indels detected. There was relatively high agreement in regions that sequence reliably, but lower concordance in regions of complexity, and there were also differences found between different variant software used (Shimmer vs Stelka). Some of the variability in results were the result of tumor and sampling heterogeneity between FF and FFPE tissues. When considering the possibility of FFPE introducing variation, it was noted that if considering all SNVs and indels and all cancer genes in the COSMIC cancer gene database, FFPE did have more mutations than FF. However, when considering only the most clinically relevant types of mutations, such as exonic and missense, > 99% of genes were not more mutated in FFPE than FF samples. Overall, 73 variants in 207 samples in 46 relevant genes were identified and verified through conformational testing using a different technology. The sensitivity of FF and FFPE were 86% and 82% respectively (Robbe et al., 2018).

Laskin et al. (2015) performed whole genome sequencing on the tumors of 100 individuals with incurable cancer, including 38 with refractory breast cancer, in the Personalized OncoGenomics (POG) study. Testing was completed in 78 patients. Of these, 55 patients received results that were considered "actionable" by a multi-disciplinary team. Twenty-three patients received treatment that was driven by WGS results. Turnaround time was a challenge, and at the beginning of the study, results took > 80 days to complete. By the end of the study, the results were completed in 50 days. The authors reported that there were limited treatment options available based on results, including even when considering available clinical trials.

## Clinical Practice Guidelines

### Professional Societies

#### American College of Obstetricians and Gynecologists (ACOG)

In the Committee Opinion 682 (2016, reaffirmed 2020), ACOG states that "the routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and

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validation studies are published." ~~ACOG and the Society for Maternal Fetal Medicine confirmed this position in 2019.~~

ACOG's 2018 Technology Assessment Number 14 addresses whole genome and whole exome sequencing, indicating that whole exome sequencing (WES) is more frequently utilized in clinical genetics, as it has greater clinical relevance and applicability to patient care. The assessment notes that when standard testing from amniocentesis or chorionic villus sampling fails to lead to a diagnosis, WES as a prenatal test may be reasonable in certain circumstances (e.g., fetuses with multiple anomalies, cases of recurrent fetal phenotypes lacking diagnosis by standard genetic tests).

American Academy of Neurology (AAN)/American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

In an updated AANEM 2016 consensus statement, the group stated that while they do not endorse or recommend a specific testing methodology, genetic testing to establish a molecular diagnosis is a crucial step in providing optimal care to individuals with neuromuscular patients disorders (Kassardjian et al., 2016).

The AAN and AANEM have indicated that there is low level evidence to consider WES or WGS in selected individuals with congenital muscular dystrophy in who a genetic variation has not been identified through standard testing approaches. Individuals with congenital muscular dystrophy that do not have causative genetic variations identified through routine methods can be considered for WES or WGS when those technologies are clinically available. Evidence Level C (Kang et al., 2015, reaffirmed September 2021).

American Society of Human Genetics (ASHG)

ASHG (Botkin et al., 2015) makes the following recommendations pertaining to the genetic testing of children and adolescents:

- Diagnostic testing:
- ⊖ Pharmacogenetic testing in children may be appropriate in the context of clear evidence of clinical utility.
- ⊖ Genetic testing should be limited to single gene or targeted gene panels based on the patient's clinical presentation when appropriate. When WGS is performed, it is ethically acceptable to limit the analysis to a limited number of genes of interest.
- ⊖ Genome-scale sequencing is appropriate when prior, more limited genetic testing has failed to identify a causative variant. Genome-scale sequencing may be appropriate as an initial genetic test under certain circumstances.

American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP)

ACMG and AMP released guidance to laboratories in 2015 on how to evaluate variations found through next generation sequencing (NGS), including WES and WGS. They also highlighted the responsibility of the ordering provider in the process, stating "due to the complexity of genetic testing, optimal results are best realized when the referring healthcare provider and the clinical laboratory work collaboratively in the testing process".

The guidelines highlight that healthcare providers need to be prepared to provide detailed information on other lab tests performed, clinical evaluations and testing, and patient phenotype. They need to understand that some results returned, such as "variants of unknown significance," may not be actionable, or the clinical implication may be

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unknown for pathogenic mutations. Testing of additional family members may be required to interpret the test results of the patient. Finally, as new data emerges, the interpretation of a variant may change over time and the healthcare provider must be prepared to monitor and manage changing interpretations. As highlighted by ACMG and AMP, "variant analysis is at present imperfect and the variant category reported does not imply 100% certainty."

#### American College of Medical Genetics and Genomics (ACMG)

In a 2021 practice guideline authored by Manickam et al., the ACMG asserts their position that evidenced-based literature supports clinical utility of whole exome and whole genome sequencing on both active and long-term management of ~~patients~~ individuals with congenital anomalies, developmental delay and intellectual disability (CA/DD/ID). Based on their comprehensive systematic review, limited evidence for negative outcomes was found. As such, the ACMG recommends use of whole exome and whole genome sequencing as a first- or second -tier test for individuals with one or more CAs with onset prior to one year of age or for individuals with DD/ID with onset prior to 18 years of age.

Malinowski et al. (2020) reported on the outcome of an ACMG systematic review performed to assist with creation of an evidence-based guideline for use of exome sequencing (ES) and genome sequencing (GS). Primary literature including health, clinical, reproductive and psychosocial outcomes resulting from ES/GS in individuals with CA/DD/ID was identified. Ultimately, 167 articles were included; these were largely case reports or small case series and, of note, all but one study lacked a comparison group. Changes to clinical management or reproductive decision-making were the most frequently reported outcomes and were observed in nearly all included studies. Further, a significant number of the articles reported clinical impact on family members of the affected individual or an impact on reproductive outcomes. The authors concluded that for individuals with CA/DD/ID, ES and GS assists with clinical and reproductive decision-making, potentially improving outcomes for ~~patients~~ affected individuals and family members. However, there were some noted conflicts of interest and the relevance of these findings on clinical outcomes is not clear.

In a 2021 ACMG policy statement, Miller et al. (2021a) published recommendations for reporting secondary findings (SF) in ES and GS. The recommendations include an SF list, which was created to provide a "minimum list" of actionable secondary findings and indicate that this list should only include genes where the clinically relevant variants are detected as part of standard clinical ES/GS. The current list, published as a policy statement of the ACMG (Miller et al., 2021b) and containing 73 genes as of v3.0 publication, describes the rationale which supports how genes are selected to be added or removed from the secondary findings list. ~~Updates to the gene list are anticipated to occur annually.~~ In 2022, Miller et al. updated the list for reporting of secondary findings in clinical ES and GS. A total of five new genes were added to the v 3.1 list including BAG3, DES, RBM20, TNNC1 (cardiomyopathy) and TTR (hereditary TTR).

Monaghan et al (2020) published a "points to consider" document on the use of fetal exome sequencing in prenatal diagnosis for ACMG. This document is meant to be used as an educational resource for clinicians. There were numerous considerations stated that span from pretest to reporting, post-test, cost, re-analysis, target family testing, and health-care professional education. The authors concluded that exome sequencing may be considered when a diagnosis cannot be obtained via routine prenatal methods in a fetus with anomalies.

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A 2019 ACMG statement (Deignan et al.) addressed points to consider in the reevaluation and reanalysis of genomic test results. Noting that the phenotype of impacted individuals may change or evolve over time and that information regarding the phenotypic spectrum of a condition and relevant related variants may also expand, this ACMG statement asserts that reanalysis is critical in the diagnostic odyssey. The document goes on to provide guidance to assist laboratories with developing policies and protocols or both variant and case level re-evaluation and reanalysis.

~~A 2019 update was made by ACMG (David et al., 2019) regarding responsibility to recontact patients when new data pertaining to genetic variants has been determined. According to the ACMG, the following should be viewed as guidance for the ordering health-care provider, clinical geneticist, laboratory geneticist, and genetic counselor:~~

- ~~• Recontact is a responsibility that is shared between the health provider, testing laboratory, and patient~~
- ~~• If the patient's medical/family history changes, the patient should notify the health provider~~
- ~~• Pregnancy and/or changes in family history including sudden death and new diagnosis in patient/relative should prompt the patient to recontact their provider to request such updates~~

~~In 2016, the ACMG released an updated policy statement on recommendations for reporting of secondary findings in clinical exome and genome sequencing. Four new genes were added to the list of recommended secondary findings, along with the elimination of one of the earlier genes from the 2013 list. The new, updated secondary findings list includes 59 medically actionable genes recommended for return in clinical genomic sequencing (Kalia et al., 2016).~~

~~The ACMG Board of Directors (2012) published a policy statement regarding use of genomic testing that recommends that WGS/WES should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:~~

- ~~• The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.~~
- ~~• A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.~~
  - ~~• A patient presents with a likely genetic disorder, but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.~~
  - ~~• A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.~~
  - ~~• WGS/WES should not be used at this time as an approach to prenatal screening.~~
  - ~~• WGS/WES should not be used as a first-tier approach for newborn screening.~~

### European Society of Human Genetics (ESHG)

Souche et al. (2022) published recommendations for use of WGS in diagnostics for rare diseases which was the result of collaboration of EuroGentest, a working group of the ESHG, and Horizon 2020 project Solve-RD which seeks to uncover genetic causes for currently unsolved rare genetic diseases using various analytical techniques. The recommendations include 44 statements which now incorporate the use of WGS, focusing on diagnostic NGS used in a clinical setting for the diagnosis of rare diseases and address many aspects of diagnostic testing including evaluation and rationale to setup of NGS

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applications including such things as quality control, variant interpretation and reporting of NGS results. General recommendations include:

- It is recommended to introduce WGS analysis in a diagnostic setting when it is a relevant improvement on quality, efficiency and/or diagnostic yield
- Diagnostic WGS for rare diseases and cancer (as well as other genetic testing approaches) should only be performed in accredited laboratories
- NGS should not be transferred to clinical practice without acceptable validation of the tests
- Confirmation, interpretation and communication to the patient of results obtained in a research setting should always be done after re-testing on (preferably) an independent sample by a diagnostic laboratory

### International Society of Prenatal Diagnosis (ISPD)

In 2022, the ISPD published an updated position statement on the use of genome-wide sequencing for prenatal diagnosis, noting the rapid increase of research and clinical use of this technology for prenatal diagnosis of fetuses at risk for genetic disorders (Van den Veyver et al, 2022). Current evidence does not support routine testing of fetal tissues obtained from an invasive prenatal procedure such as amniocentesis or chorionic villus sampling (CVS) in the absence of fetal anomalies. The position statement indicates there is data to support benefit of prenatal sequencing for the following:

- Current pregnancy where fetus has a major single anomaly or multiple organ system anomalies and;
  - No genetic diagnosis found after CMA and genetic expert considers the phenotype suggestive of genetic etiology
  - Multiple anomaly pattern strongly suggests a single gene disorder with no prior genetic testing. CMA should be run before in parallel with prenatal exome sequencing (pES) in this case.
- Personal history of prior undiagnosed fetus or child with a major single or multiple anomalies and;
  - Recurrence of similar anomalies in current pregnancy without genetic diagnosis after karyotype or CMA for current or prior undiagnosed pregnancy.
  - When parents present for preconception counseling and no sample is available from the affected proband, or if a fetal sample is unable to be obtained in ongoing pregnancy, sequencing may be offered for both biological parents to look for shared carrier status of autosomal recessive mutations that could explain phenotype. Tissue from previous abnormal fetus/child for pES is preferable.
  - In special circumstances, consideration of testing may be given in circumstances where it would not normally be advised, such as strong family history of recurrent childhood-onset severe genetic condition in specific circumstances, but these should be reviewed by an expert multi-disciplinary team, most appropriately in the context of a research protocol.

~~International Society of Prenatal Diagnosis (ISPD), the Society of Maternal Fetal Medicine (SMFM) and the Perinatal Quality Foundation (PQF) (2018)~~

~~In this joint position statement, the ISPD, SMFM and PQF note that routine diagnostic sequencing of the fetus cannot be supported due to insufficient validation data. It is recommended that a research setting where testing is offered on a case-by-case basis is the best current approach. However, there are some limited scenarios where testing may be beneficial:~~

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- A current pregnancy with a single major anomaly or the multiple anomalies suggesting of a genetic etiology
- Chromosome microarray is negative
- A maternal and/or paternal history of a previous pregnancy with similar anomalies with no diagnosis
- A family history of recurrent stillbirths of unknown etiology

~~There is no indication for testing in the absence of fetal anomalies.~~

### **National Society of Genetic Counselors (NSGC)**

In a 2022 evidence-based practice guideline, the NSGC (Smith et al.) provided recommendations regarding the use of genetic testing for individuals with epilepsy, noting that a majority of unexplained epilepsy is estimated to have an underlying genetic etiology. The recommendations are as follows:

- Genetic testing with exome/genome sequencing and/or a multi-gene panel (>25 genes) is strongly recommended for all individuals with unexplained epilepsy, regardless of age, as first-tier testing, followed by chromosomal microarray. Exome/genome sequencing is conditionally recommended over multi-gene panel.
- It is strongly recommended that genetic tests be selected, ordered, and interpreted by a qualified healthcare provider in the context of appropriate pre- and post-test genetic counseling.

~~In a 2017 position statement, NSGC endorsed multi-gene panel use when clinically warranted. They recommended that physicians evaluate the analytical/clinical validity and clinical utility of genetic testing methodologies prior to ordering. Family history, clinical/medical records, gene panel content, variant interpretation/reporting practices and the limitations of sequencing technologies should also be taken into consideration (NSGC, 2017).~~

## **U.S. Food and Drug Administration (FDA)**

This section is to be used for informational purposes only. FDA approval alone is not a basis for coverage.

Laboratories that perform genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. More information is available at: <https://www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm>.

~~(Accessed November 28, 2018)~~

**(Accessed October 17, 2022)**

No FDA-approved tests for WES or WGS are available at this time.

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## Policy History/Revision Information

| Date       | Summary of Changes   |
|------------|--|
| <u>TBD</u> | <p><u>Coverage Rationale</u></p> <p><u>State-Specific Criteria</u></p> <ul style="list-style-type: none"> <li><u>Added language to indicate the coverage criteria for genetic counseling contained in this policy represents Louisiana Medicaid Managed Care Organization Manual (LA MCO) coverage policy and is set forth [in the policy] in accordance with State requirements</u></li> </ul> <p><u>Genetic Counseling</u></p> <ul style="list-style-type: none"> <li><u>Revised language to indicate genetic counseling before and after all genetic testing is required; counseling must consist of at least all of the following and be documented in the medical record:</u> <ul style="list-style-type: none"> <li><u>Obtaining a structured family genetic history; and</u></li> <li><u>Genetic risk assessment; and</u></li> <li><u>Counseling of the enrollee and family about diagnosis, prognosis, and treatment</u></li> </ul> </li> </ul> <p><u>Additional Non State-Specific Criteria</u></p> |

### Whole Genome Sequencing (WGS)

- Revised coverage criteria:
  - Added criterion requiring:
    - Clinical history strongly suggests a genetic cause and one or more of the following features are present:
      - Multiple congenital anomalies (must affect different organ systems)
      - Moderate, severe, or profound Intellectual Disability diagnosed by 18 years of age
      - Global Developmental Delay
      - Epileptic encephalopathy with onset before three years of age
    - Clinical history strongly suggests a genetic cause and two or more of the following features are present:
      - Congenital anomaly
      - Significant hearing or visual impairment diagnosed by 18 years of age
      - Laboratory abnormalities suggestive of an inborn error of metabolism (IEM)
      - Autism spectrum disorder
      - Neuropsychiatric condition (e.g., bipolar disorder, schizophrenia, obsessive-compulsive disorder)
      - Hypotonia or hypertonia in infancy
      - Dystonia, ataxia, hemiplegia, neuromuscular disorder, movement disorder, or other neurologic abnormality
      - Unexplained developmental regression, unrelated to autism or epilepsy
      - Growth abnormality (e.g., failure to thrive, short stature, microcephaly, macrocephaly, or overgrowth)
      - Persistent and severe immunologic or hematologic disorder
      - Dysmorphic features
      - Consanguinity
      - Other first- or second-degree family member(s) with similar clinical features
    - Reanalysis of WES after at least 18 months when criteria [listed in the policy] for initial WES has been met and one of the following occurs:
      - Individual experiences additional symptoms after initial WES that cannot be explained by the results of the initial WES; or
      - New data or new family history emerges which suggest a link between the individual's symptoms and specific genes
  - Removed criterion requiring:
    - Clinical presentation or clinical and family history strongly suggests a genetic cause for which a specific clinical diagnosis cannot be made with any clinically available targeted genetic tests
    - There is a clinical diagnosis of a genetic condition where there is significant genetic heterogeneity and WES is a more practical approach to identifying the underlying genetic cause than are individual tests of multiple genes

- There is likely a genetic disorder and multiple targeted gene tests that have failed to identify the underlying cause
- Replaced criterion requiring "WES is ordered by a board-certified medical geneticist, neonatologist, neurologist, or developmental pediatrician" with "WES is ordered by a medical geneticist, neonatologist, neurologist, or developmental pediatrician"
- Removed language indicating further studies are needed to evaluate the clinical utility of WES for other indications [not listed in the policy]

#### Whole Genome Sequencing (WGS)

- Added language to indicate Whole Genome Sequencing (WGS) is Medically Necessary for the following:
  - Diagnosing or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes and all of the following criteria are met:
    - Neither chromosome microarray analysis (CMA) nor WES have been performed; and
    - Clinical presentation is nonspecific and does not fit a well-defined syndrome for which a specific or targeted gene test is available; if a specific genetic syndrome is suspected, a single gene or targeted gene panel should be performed prior to determining if WGS is necessary; and
    - WGS is ordered by a medical geneticist, neonatologist, neurologist, or developmental pediatrician; and
    - One of the following:
      - Clinical history strongly suggests a genetic cause and one or more of the following features are present:
        - Multiple congenital anomalies (must affect different organ systems)
        - Moderate, severe, or profound Intellectual Disability diagnosed by 18 years of age
        - Global Developmental Delay
        - Epileptic encephalopathy with onset before three years of age
      - Clinical history strongly suggests a genetic cause and two or more of the following features are present:
        - Congenital anomaly
        - Significant hearing or visual impairment diagnosed by 18 years of age
        - Laboratory abnormalities suggestive of an IEM
        - Autism spectrum disorder
        - Neuropsychiatric condition (e.g., bipolar disorder, schizophrenia, obsessive-compulsive disorder)
        - Hypotonia or hypertonia in infancy
        - Dystonia, ataxia, hemiplegia, neuromuscular disorder, movement disorder, or other neurologic abnormality
        - Unexplained developmental regression, unrelated to autism or epilepsy
        - Growth abnormality (e.g., failure to thrive, short stature, microcephaly, macrocephaly, or overgrowth)

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- Persistent and severe immunologic or hematologic disorder
- Dysmorphic features
- Consanguinity
- Other first- or second-degree family member(s) with similar clinical features
- Comparator (e.g., parents or siblings) WGS for evaluating a genetic disorder when the [listed] criteria have been met and WGS is performed concurrently or has been previously performed on the individual
- Replaced language indicating "WGS is unproven and not Medically Necessary for screening and evaluating any disorder" with "WGS is not Medically Necessary for any other clinical situation [not listed in the policy as proven and medically necessary] due to the availability of clinically equivalent diagnostic tests"
- Removed language indicating:
  - Although WGS has the potential to identify causal variants for a wide variety of conditions that may be missed with other technologies, as well as to identify predictive biomarkers, the information derived from WGS has not yet been translated into improved outcomes and changed medical management
  - Further studies are needed to establish the clinical utility of WGS
- Added notation to indicate this policy is limited to genetic testing in an outpatient setting or upon discharge from an inpatient setting; the evaluation of cancer is addressed in the Medical Policy titled *Molecular Oncology Testing for Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only)*

#### Definitions

- Added definition of:
  - Consanguinity
  - Global Developmental Delay
  - Intellectual Disability
  - Medically Necessary

#### Applicable Codes

- Added CPT codes 0212U, 0213U, 0214U, and 0215U
- Added notation to indicate CPT codes 0036U, 0094U, 0212U, 0213U, 0214U, 0215U, 0265U, 0335U, 0336U, 81415, 81416, 81417, 81425, 81426, and 81427 are not on the State of Louisiana Fee Schedule and therefore may not be covered by the State of Louisiana Medicaid Program

#### Supporting Information

- Updated *Description of Services, Clinical Evidence, and References* sections to reflect the most current information
- Archived previous policy version CS150LA.H



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## Instructions for Use

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