

Whole Exome and Whole Genome Sequencing (for Louisiana Only)

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[Instructions for Use](#)

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Application

This Medical Policy only applies to the state of Louisiana.

Coverage Rationale

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.

Whole Exome Sequencing (WES)

Whole Exome Sequencing (WES) is proven and medically necessary for diagnosing or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes and all of the following 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:
 - The clinical presentation or clinical and family history strongly suggest a genetic cause for which a specific clinical diagnosis cannot be made with any clinically available targeted genetic tests; or
 - 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; or
 - There is likely a genetic disorder and multiple targeted gene tests that have failed to identify the underlying cause

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.

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

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

Whole Genome Sequencing (WGS)

Whole Genome Sequencing (WGS) is unproven and not medically necessary for screening and evaluating any disorder. 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 patient's DNA sequence. This may be a parent or sibling of the patient, or non-cancerous tissue that is being compared to the patient's tumor tissue (Thun et al., 2017).

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).

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).

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
0012U	Germline disorders, gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood, report of specific gene rearrangement(s)

CPT Code	Description
0013U	Oncology (solid organ neoplasia), gene rearrangement detection by whole genome next-generation sequencing, DNA, fresh or frozen tissue or cells, report of specific gene rearrangement(s)
0014U	Hematology (hematolymphoid neoplasia), gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood or bone marrow, report of specific gene rearrangement(s)
0036U	Exome (i.e., somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses
0094U	Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis
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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Description of Services

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). 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).

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, What are whole exome sequencing and whole genome sequencing? 2018). As with WES, WGS results in long lists of unknown variants, and 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., 2015).

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

Pediatric (Non-Cancer)

Stark, 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, and included lifesaving treatment for a 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 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, 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 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. The authors hypothesize that WES at the first indication of a genetic disorder would have reduced the number of tests and interventions, and provided an overall cost savings.

Vissers 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-Graovac et al. (2016) combined deep clinical phenotyping (the comprehensive characterization of the discrete components of a 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.

Retterer 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.

Valencia 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 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.

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 consecutive patients with clinical WES analyzed between June 2012 and August 2014. WES tests were performed at a clinical 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 physician. The patients were primarily pediatric (1756 [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 Mendelian disease patterns of the 527 molecular diagnoses included 280 (53.1%) autosomal dominant, 181 (34.3%) autosomal 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 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%.

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 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.

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.

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 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.

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.

WES Adult (Non-Cancer)

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 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 γ* , *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 median 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 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 γ* 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 hypervariation 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.

Whole Genome Sequencing

WGS-Pediatric (Non-Cancer)

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 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 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.

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 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 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, 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 NIH Rare Disease 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 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 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

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.

Professional Societies

American College of Obstetricians and Gynecologists (ACOG)

In the Committee Opinion 682 (2016), 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 validation studies are published.”

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

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).

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 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 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.

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)

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
04/01/2021	Template Update <ul style="list-style-type: none"> Removed <i>Related Policies</i> and <i>CMS</i> sections Updated <i>Instructions for Use</i>; replaced reference to “MCG™ Care Guidelines” with “InterQual® criteria”
02/01/2021	Template Update <ul style="list-style-type: none"> Reformatted policy; transferred content to new template

Date	Summary of Changes
10/01/2019	<ul style="list-style-type: none"> Created state-specific policy version for Louisiana (no change to guidelines)
07/01/2019	<p>Application</p> <ul style="list-style-type: none"> Added language to indicate this policy does not apply to the state of Tennessee; refer to the Medical Policy titled Whole Exome and Whole Genome Sequencing (for Tennessee Only) <p>Applicable Codes</p> <ul style="list-style-type: none"> Updated list of applicable CPT codes to reflect quarterly code edits; added 0094U <p>Supporting Information</p> <ul style="list-style-type: none"> Archived previous policy version CS150.E

Instructions for Use

This Medical Policy provides assistance in interpreting UnitedHealthcare standard benefit plans. When deciding coverage, the federal, state or contractual requirements for benefit plan coverage must be referenced as the terms of the federal, state or contractual requirements for benefit plan coverage may differ from the standard benefit plan. In the event of a conflict, the federal, state or contractual requirements for benefit plan coverage govern. Before using this policy, please check the federal, state or contractual requirements for benefit plan coverage. UnitedHealthcare reserves the right to modify its Policies and Guidelines as necessary. This Medical Policy is provided for informational purposes. It does not constitute medical advice.

UnitedHealthcare may also use tools developed by third parties, such as the InterQual® criteria, to assist us in administering health benefits. The UnitedHealthcare Medical Policies are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.