Genomic Sequencing:

An Evolving Standard in Molecular Genetic Diagnosis

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Abstract

Genetic diagnostic testing has developed at an explosive pace. Examples of groundbreaking technologies that have evolved in recent decades include cytogenetic techniques (karyotype analysis, fluorescence in situ hybridization [FISH], and chromosomal microarray analysis [CMA]), restriction fragment length polymorphism (RFLP) analysis, multiplex ligation-dependent probe amplification (MLPA), Sanger sequencing, and next generation sequencing (NGS) techniques. Appropriately utilizing and integrating the various available genetic testing technologies into clinical care has challenged even the most specialized practitioners. Hand in hand with these technologic advances, clinical molecular genetic testing strategies have undergone a revolution. Whereas at one time, single gene analysis was the primary testing approach, targeted panel-based testing rapidly developed, giving way more recently to comprehensive sequencing methodologies, such as whole exome sequencing (WES) and whole genome sequencing (WGS).

Comprehensive sequencing continues to gain rapid ground as a diagnostic testing strategy. Recognition and confirmation of the diagnostic success rates of genomewide sequencing have led to the development of numerous guidelines recommending its use as a first-tier genetic diagnostic test in many scenarios.[1,2,3] Increased coverage of genomic sequencing technologies by third-party payors and expanding clinical adoption have dramatically decreased the time to diagnosis, as well as the overall expense of testing. The arena of prenatal genetics (including noninvasive prenatal diagnosis), in which the technologies have not yet been universally adopted, is a new horizon for application of comprehensive sequencing. It is important that clinicians be aware of the diagnostic value of genomic testing technologies and have sufficient knowledge regarding their recommended use. Third-party payors should continue to be urged to expand reimbursement for this testing in those clinical scenarios in which it is appropriate.

Introduction

Time to diagnosis of complex congenital and/or developmental disorders influences care planning, and if prolonged, may disadvantage patients needing specialized or timely treatment, at times even impacting survival.[1] The importance of a diagnosis is evidenced in aspects of patient care such as determining prognosis, providing accurate recurrence risk estimates, facilitating preimplantation and prenatal diagnosis, initiating appropriate disease surveillance measures, and planning other specialized medical management.[1] The significance of a definitive diagnosis for the psychological adaptation of the patient/family cannot be underestimated.

Use of single gene or panel testing in the absence of clear clinical indicators for a targeted approach (such as evidence for chromosomal trisomy or a clearly defined genetic syndrome) can delay the discovery of information that may be important for clinical decision-making. This is because the additive turnaround time of multiple tests performed in a sequential manner (an approach required when initial testing fails to yield an answer) can significantly prolong the time to a definitive result, compared with performing one highly comprehensive test as a first-tier approach; in addition, the cost of multiple tests may be significantly higher overall. Comprehensive testing modalities are important to consider in these cases, as comprehensive sequencing tests can detect several classes of molecular sequence variants, as opposed to only one or select genes (as with single gene or targeted gene panel analysis). The exome makes up 1% of the human genome and has the highest likelihood of harboring disease-causing variants.[3] In the case of WGS, detection of copy number variants (CNVs), mitochondrial variants, and intronic and noncoding elements increases the overall diagnostic yield. Predictably, the cost of WGS is higher than WES at the current time; however, new sequencing technologies are rapidly changing this landscape.

Studies have shown an overall diagnostic yield for WES of 38%.[4] The added yield of WGS over WES may be as high as 10% to 20%[2,5] (Table 1). The sensitivity of this testing is optimized when performed as a trio of tests (i.e., samples from the proband and both parents are analyzed), as this allows determination of the biologic parent of origin of biallelic variants and distinguishing de novo variants. Given the high detection rates observed, the American College of Medical Genetics and Genomics (ACMG)[3] strongly recommends WES or WGS as a first- or second-tier test for patients with congenital anomalies or developmental delay/intellectual disability.[2]

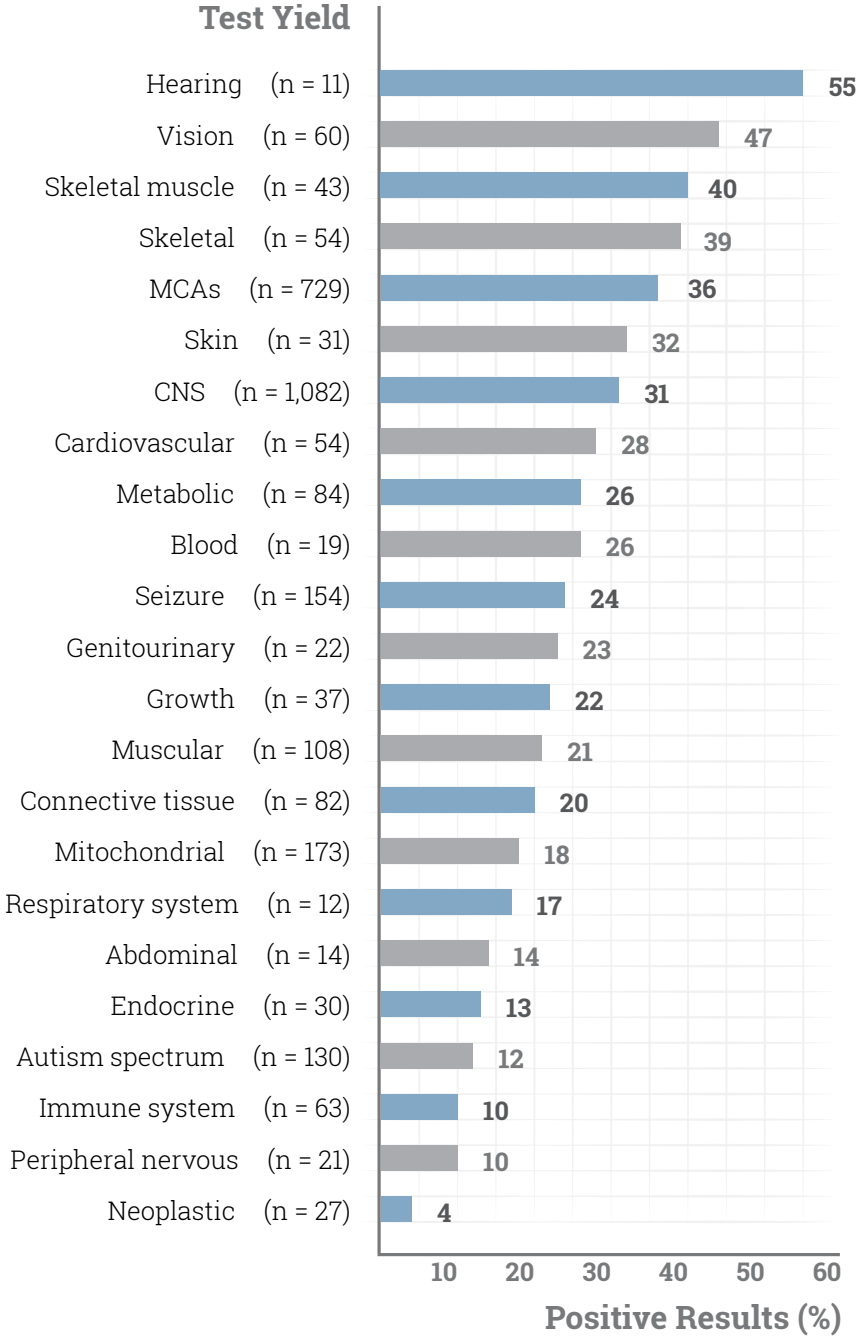
Table 1. Diagnostic yield of WES and WGS

Genomic Sequencing Approach	Diagnostic Yield
WES	34%-38%
WGS	43%-52%

Sources: Manickam, 2021[2]; Retterer, 2016[4]; Ewans, 2022[5]

Examining the clinical yield of WES by clinical indication is informative. Studies of WES have identified that clinical indications that show the highest detection rates are neurologic conditions (specifically hearing and vision, with yields of 55% and 47%, respectively)[4] (Figure 1). Additional detection rates of note include 39% for skeletal defects, 36% for multiple congenital anomalies (MCAs), and 31% for central nervous system (CNS) disorders.[4] A recent meta-analysis found that the diagnostic yield was 30.6% for WGS and 23.2% for WES in pediatric patients with rare and undiagnosed genetic diseases.[6] These findings emphasize the value of genome sequencing-based technologies as the most comprehensive diagnostic testing methodologies available today. It should be stressed that clinical evaluation by appropriate specialists should always be a component of patient assessment. Involvement of a medical geneticist at any point in the process is appropriate when a clinician's comfort level or confidence in ordering these advanced sequencing tests is low.[1]

Figure 1. Cases with definitive diagnosis by phenotype.



(Adapted from Retterer, 2016[4])

In developmental delay (DD), intellectual disability (ID), and autism spectrum disorders, the diagnostic yield using WES is 28% to 43% (estimates do not yet exist for WGS).[1] This has resulted in the recent adjustment of American Academy of Pediatrics recommendations for genomic sequencing to consider use of WES as a first-tier testing approach.[1] The genetic workup in developmental delay (DD)/intellectual

disability (ID) should include a thorough, systematic evaluation including clinical exam, detailed family and medical histories, and other indicated investigations, such as medical imaging or hearing and vision assessments.[1]

Rapid exome or genome sequencing, for which results are often available within a few days, can be considered when available and when results will have an immediate impact on care decisions, such as in the neonatal intensive care unit (NICU) setting, where results may be a matter of life or death. Although considerably more costly, rapid genome sequencing can be an important consideration in acute clinical presentations. In these cases, results of rapid testing may obviate other expensive diagnostic testing, as well as potentially therapeutic and/or surveillance modalities with undesirable side effects, including invasive procedures.[2]

Important limitations of genomic sequencing technologies include the inability to diagnose methylation defects or trinucleotide repeat disorders (although detection in this latter category is improving). Additionally, the coverage of given regions of the genome may be poor, and detection of small deletions may be limited. Importantly, laboratory test results interpretation relies heavily on the phenotypic information provided.[1] In general, provision of a specific and thorough clinical history as well as phenotypic findings/features improves the likelihood of correctly identifying causative pathogenic or likely pathogenic variants. It is important that the performing laboratory have extensive experience and expertise in variant interpretation to optimize the diagnostic yield of genomic sequencing results.

The offer of genomic sequencing technologies requires appropriate patient and family education and informed consent. Considerations such as cost and insurance coverage and the possibility of uncovering variants of uncertain significance (VUSs) or incidental findings, and in rare cases, nonbiologic relationships, are important to discuss before testing. The option of reporting secondary findings (findings considered medically actionable, for which it is agreed receipt of results should be an option) should be carefully addressed. Secondary findings can inform patients of presymptomatic conditions, such as cancer or cardiovascular disease risk. Implications from the reporting of such variants may include psychological distress and impact on insurability.[1] The recommended list of reportable secondary findings is reviewed and updated annually by ACMG.[7]

Numerous laboratories offer prenatal genomic sequencing testing. The utility of this testing is gaining visibility in the setting of complex fetal malformations or structural anomalies. A genetic fetal diagnosis can enable prognostication and prenatal and perinatal management planning and may result in more favorable clinical outcomes. The indirect nature of the fetal evaluation (ultrasound, screening tests, etc.) can pose a particular challenge in pinpointing specific genetic conditions for targeted testing in the prenatal setting, which makes genomewide sequencing approaches appealing. Conversely, it can be difficult to identify adequate phenotypic information for the most accurate results interpretation due to factors such as the quality of imaging equipment, gestational age, fetal positioning, and maternal body habitus or scanning characteristics.[8] In addition, the fetal phenotypes of rare conditions may not be well described. The yield of fetal WES has been demonstrated to be 10% to 30% after a normal karyotype and microarray.[8,9] Pre- and posttest counseling are highly important for informed decision-making in the context of prenatal genomic sequencing, given its complexity.

An increasing number of reports are confirming remarkable reductions in time to diagnosis with utilization of WES or WGS technologies. A recent study[10] outlined improvements conferred by a policy change allowing rapid WES or rapid WGS as first-line testing in pediatric inpatients who underwent a genetics consultation. Forty-two percent of general pediatric patients received a diagnosis through exome or genome sequencing. The researchers demonstrated a remarkable reduction in average time to diagnosis, from 9.5 months to slightly less than two weeks.[10] Studies of the cost-effectiveness of WES- or WGS-based testing are in process, and preliminary evidence points to reduced costs, greater clinical impact, and improved patient care.[10,11,12]

Research

The results of testing using WES/WGS have contributed to the knowledge base regarding expected outcomes from discovered genetic variants and expanded understanding of the broad scope of disease expression, the natural history of disorders and disease processes, and potential treatments.[2] This growth in understanding has increased diagnostic yield and may in time contribute to the advancement of new technologies such as gene therapy and gene editing.[2] There may be future benefits such as contributions toward defining the clinical spectrum for lethal disorders, for which the full phenotype may not yet be understood.

Conclusions

The value and impact of genomic sequencing has become clear. Without a doubt, evidence now confirms increased clinical utility conferred by a higher diagnostic yield with the use of genomic sequencing testing technologies. More patients can benefit from the personalized care management enabled by comprehensive sequencing results.[6] The importance of educating third-party payors regarding the value and importance of plan coverage for comprehensive sequencing technologies in certain clinical scenarios cannot be overemphasized.

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