Washington State Health Care Authority

FINAL Key Questions and Background

Whole Exome Sequencing

Background

Whole exome sequencing (WES) may be applicable to testing for a wide range of genetic disease. It is most commonly used when a disorder is suspected to be genetic but is not recognizable clinically or when the patient's symptoms are consistent with a wide range of genetic disorders. Experts recommend a family physician consider that a condition may be genetic when a patient has any of the following: dysmorphic features, multiple anomalies, unexplained neurocognitive impairment, or a family history suggestive of a genetic disease.[1] Other signs of a potential genetic disorder include a much earlier onset of symptoms than is common, a multifocal presentation (i.e., bilateral cataracts, many colon polyps, etc.; or an unusual combination of symptoms).[2] Some conditions with pediatric onset may not be diagnosed in childhood, leading to adult patients who may present with a confusing mix of symptoms.[3]

WES identifies the DNA base pair sequence of the protein coding regions of the genome, including proximal regulatory segments and the splicing junctions.[4] WES is primarily used to identify small changes in base pair sequences that disrupt protein function and cause disease, but new bioinformatics software has increased the ability to identify chromosomal copy number variants (i.e., larger deletions or duplications involving larger stretches of DNA) from sequenced data. WES may be done for clinical or research purposes. Diagnostic WES testing is ordered by a physician or other health care professional and is conducted in a clinical diagnostic laboratory to aid in the diagnosis of a patient. The proband's parents or siblings may be sequenced to help interpret identified variants. Research WES testing is used to identify and characterize a common disease gene or genes among multiple families or patients with a similar phenotype.

WES uses next generation sequencing (NGS) technologies, which makes many copies of the target genome, cuts them into random sequences, and then simultaneously sequences the resulting fragments. WES requires multiple layers of bioinformatics analysis, often referred to as the analysis pipeline.[5] This pipeline includes identifying variants in the sequenced genome against a reference genome, identifying the gene in which the variant occurs and its function, classifying variants as pathogenic (or not) in relationship to the patient's clinical phenotype, and reporting all variants identified that are associated with the clinical phenotype along with other American College of Medical Genetics and Genomics (ACMG)-defined medically actionable findings in genes not associated with the patient's clinical phenotype. Most laboratories allow patients to opt-out of receiving medically actionable findings or other secondary findings.

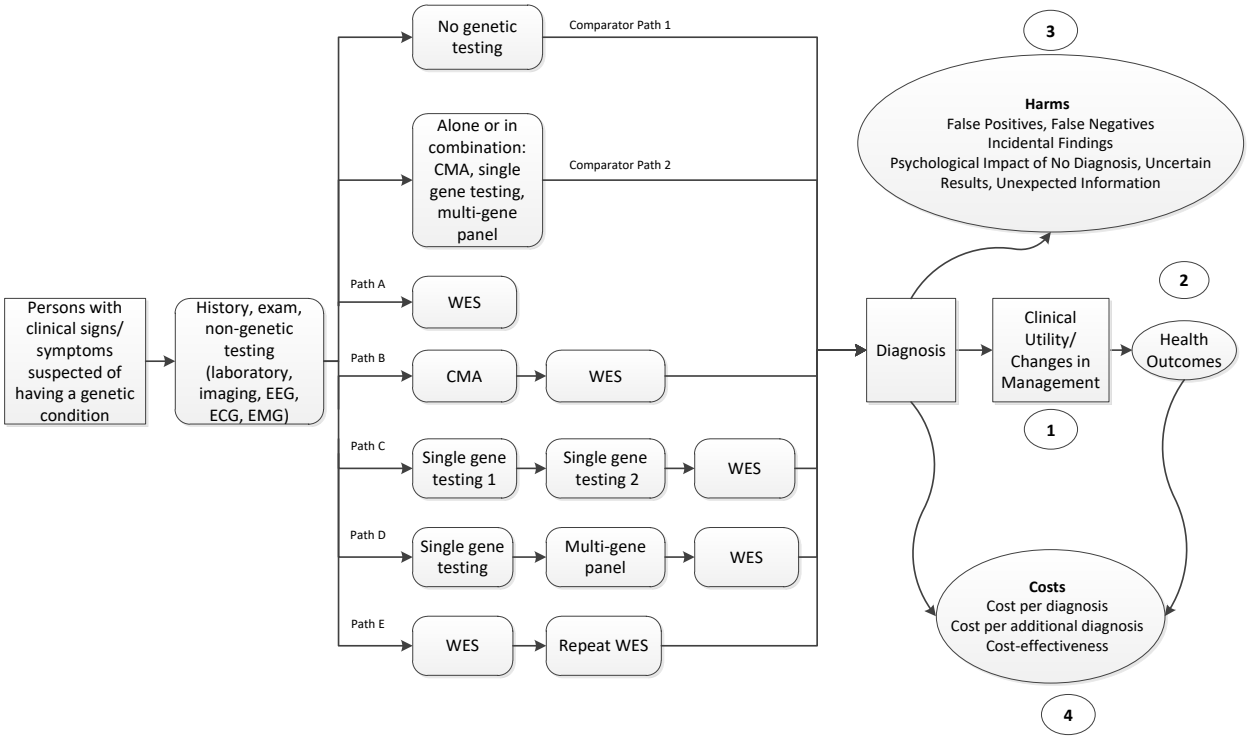
Policy Context

The State of Washington Health Care Authority selected WES as a topic for a health technology assessment because of high concerns for safety and medium concerns for efficacy, and cost.

Scope of this HTA

The analytic framework (***Figure 1***), research questions, and study selection criteria are listed in this section.

Figure 1. Analytic Framework Depicting Scope of Proposed Health Technology Assessment



Key Question 1: Effectiveness (Clinical Utility)

1a. In what proportion of patients does testing with WES result in a clinically actionable finding (i.e., the diagnosis resulting from WES leads to something that can be treated, prevented, or mitigated)?

1b. In what proportion of patients does testing with WES result in an actual change to the patient's medical management (medication or therapies, follow-up testing, medical monitoring) or genetic counseling (reproductive risks or risks of other family members)?

1c. What is the effect of testing pathways that include WES on medical management or genetic risk counseling compared to testing pathways that do not include WES?

Key Question 2: Effectiveness (Health Outcomes)

2a: What are the health outcomes, including mortality, among patients who have WES testing?

2b: What are the health outcomes, including mortality, of patients who receive testing pathways that include WES compared to alternative testing pathways with or without WES?

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Key Question 3: Safety and Harms

3a: How many patients receive erroneous results after WES testing, either false positive or false negative results? What harms are caused by these test results and how many patients experience these harms?

3b: What harms are caused by uncertain WES results or a lack of diagnosis after WES testing?

3c: How many patients receive reports on ACMG-defined medically actionable variants after WES testing? What harms do they experience, and how many patients experience these harms?

3d: How frequently do WES results cause harm to family relationships?

Key Question 4: Cost

4a: What is the cost of WES testing?

4b: What is the cost per diagnosis of pathways that include WES testing?

4c: What is the cost per additional diagnosis, comparing a pathway with WES to an alternative pathway with or without WES?

4d: What is the cost-effectiveness of testing with WES?

Contextual questions will not be systematically reviewed and are not shown in the analytic framework. To address contextual questions, we will rely on recent systematic reviews and/or a subset of the largest, most recent primary research articles identified through our search.

Contextual Question 1: What is the diagnostic yield of WES either alone or as part of a testing pathway and what are the factors (e.g., phenotypes being tested, testing platforms and bioinformatics analysis used) that contribute to variation in diagnostic yields?

Contextual Question 2: How often does WES return variants of uncertain clinical significance and what impact does repeat bioinformatics analysis have on diagnostic yield?

Table 1 provides the study selection criteria we will use to select studies for inclusion in this HTA; these criteria are organized by population, intervention, comparator, outcomes, timing, setting, and study design and risk of bias criteria.

Table 1. Proposed Population, Intervention, Comparator, Outcome, Timing, and Setting for HTA on Whole Exome Sequencing

Domain	Included	Excluded
Population	Children or adults, with or without a clinical diagnosis, suspected of having a genetic disease	• Embryos and fetuses • Patients with nonsyndromic cancer or infections, where WES is being used to characterize the tumor or microbe • Deceased persons
Intervention	• Diagnostic WES alone (Path A in Figure 1) or as part of a sequential testing pathway after clinical, laboratory and imaging evaluation (Path B, C, D in Figure 1) • Re-analysis of diagnostic WES findings at a later interval (Path E in Figure 1)	• Single gene sequencing (traditional Sanger sequencing or next generation sequencing) • Multi-gene panels (traditional Sanger sequencing or next generation sequencing) • Whole mitochondrial sequencing • WES to identify acquired mutations in tumors • WES of infectious agents • Genome-wide association studies • Research-based WES (i.e., studies focused on elucidating the biology or underlying genetics of a disorder) • WES when focused on evaluating alternative methods for sequencing or variant calling • WES when focused exclusively on identifying copy number variants • Whole genome sequencing
Comparator	• Clinical, laboratory, or imaging evaluation with no genetic testing (Comparator Path 1 in Figure 1) • Testing pathways that use only CMA, single gene testing, or multigene panels (Comparator Path 2 in Figure 1). Single gene testing and multigene panels can be performed by traditional Sanger sequencing or with next generation sequencing. • Testing pathways that use WES in sequence with other testing, and including WES reanalysis (Path B, C, D, and E in Figure 1).	• Whole genome sequencing

Outcomes	• Clinical utility
	  ○ Results from WES could be or are used for medical management (e.g. therapy, further diagnostic testing, monitoring), reproductive counseling, or risk counseling for other family members
	• Health outcomes
	  ○ Mortality, length of survival
	  ○ Morbidity, cognitive ability, functional outcomes
	• Safety
		• Outcome differences due only to different genetic defects
		• Clinical utility and health outcomes related to incidental findings
		• Cost of testing from studies performed in non-U.S. countries
		• Cost of testing from studies performed in the U.S. but that are older than 2 years.

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Domain	Included	Excluded
	○ Misdiagnosis (false positives, false negatives) ○ Proportion of patients with ACMG-defined medically actionable variants ○ Psychosocial harms (e.g., anxiety, family stress, depression, distress, financial consequences) to proband and family from testing related to lack of diagnosis, uncertain findings, incidental findings, and unexpected information (e.g., carrier status, non-paternity) ○ Employment or insurance Discrimination • Costs ○ Cost of testing (U.S. based studies from previous 2 years only) ○ Cost per diagnosis ○ Cost per additional diagnosis ○ Cost-effectiveness	
Setting	Any outpatient or inpatient clinical setting in countries categorized as 'very high' on the UN Human Development Index	Non-clinical settings, countries categorized other than 'very high' on the UN Human Development Index
Study Design and Risk of Bias Rating	Study designs[6] • Clinical trial (single group or controlled) • Cohort (single group of more than 10 participants or families or controlled) • Case-control • Cross-sectional • Case series (between 5 to 10 participants or families) • Cost analyses, cost-benefit analysis, cost utility analysis, cost-effectiveness analysis • Modeling studies (for clinical utility, health outcomes, and cost outcomes only) • Qualitative study designs (for safety outcomes only) Risk of Bias Rating • Any	• Case reports (fewer than 5 participants) • Narrative reviews • Editorials and commentary • Letters to the editor
Language and Time Period	• English • 2010 or later	• Any language other than English • Studies published prior to 2010

Abbreviations: CMA=chromosomal microarray analysis; HTA=health technology assessment; WES=whole exome sequencing; UN=United Nations

Notes: [a]Andorra, Argentina, Australia, Austria, Bahrain, Belgium, Brunei Darussalam, Canada, Chile, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hong Kong China (SAR), Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea (Republic of), Kuwait, Latvia, Liechtenstein, Lithuania, Luxembourg,

Malta, Montenegro, Netherlands, New Zealand, Norway, Poland, Portugal, Qatar, Romania, Russian Federation, Saudi Arabia, Singapore, Slovakia, Slovenia, Spain, Sweden, Switzerland, United Arab Emirates, United Kingdom, United States.

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Public comment and response

See **Draft key questions: Comment and response** document published separately.

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