

Points to consider in the clinical application of genomic sequencing

ACMG Board of Directors

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

DEFINITIONS

The following definitions are used in this policy statement:

Next-generation sequencing

This term encompasses a variety of technologies that permit rapid sequencing of large numbers of segments of DNA, up to and including entire genomes. Massively parallel sequencing (also called next-generation sequencing), therefore, is not a test in itself or a specific sequencing technology. The term emphasizes a distinction from initial approaches that involved sequencing of one DNA strand at a time.

Whole-genome sequencing

This term implies the determination of the sequence of most of the DNA content comprising the entire genome of an individual. In fact, however, there may be components of the genome that are not included in a present-day “whole-genome sequence (WGS).”

Whole-exome sequencing

The “exome” is the component of the genome that predominantly encodes protein; these segments are referred to as “exons” and can include noncoding exons. The exome comprises about 1% of the genome and is, so far, the component most likely to include interpretable mutations that result in clinical phenotypes. Whole exome sequencing (WES) involves determination of the DNA sequence of most of

these protein-encoding exons and may include some DNA regions that encode RNA molecules that are not involved in protein synthesis. WES offers lower-cost analysis than WGS. It is possible that some clinically significant mutations may be missed by this approach due to inefficient capture of certain exons. In some cases, exome testing or analysis may be targeted to particular genes of clinical interest for a given application.

Overall analytical sensitivity (due, for example, to incomplete exome capture or systematic errors that are platform specific) is still being defined for both WES and WGS.

This Policy Statement was developed primarily as an educational resource for clinical and laboratory geneticists to help them provide quality clinical and laboratory genetic services. Adherence to these Points to Consider is voluntary and, in determining the relevance of and weight to be given to any specific point, the clinical and laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen.

POLICY STATEMENT

The ACMG recognizes that genomic sequencing approaches can be of great value in the clinical evaluation of individuals with suspected germ-line genetic disorders. Although this is an area that will continue to evolve with further research and technological development, there are already instances in which genomic sequencing approaches can and should contribute to clinical care.

Large-scale sequencing generates a variety of heterogeneous results. In many cases, the results will provide an explanation for a patient’s phenotype by identifying a mutation in a gene known to be associated with the patient’s clinical condition or in a gene that is highly likely to be causative given current knowledge. Such “diagnostic results” are qualitatively different from another class of results that will be regularly generated when employing these techniques: secondary findings (also called incidental or unanticipated findings). Such secondary findings are highly likely, if not inevitable, whenever WGS/WES is performed. Examples include the finding of a previously unsuspected high risk of disease in the future or the

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discovery of an as yet clinically unrecognized disorder in an asymptomatic individual. This latter type of result is similar to the types of results generated when screening an asymptomatic individual.

The following points should be considered when applying these approaches clinically for diagnosis or screening (as distinguished from primarily research-based applications of genomic technologies). When interpreting secondary findings, or results that are generated in the course of screening asymptomatic individuals, it is critical that the standards for what is reportable be high to avoid burdening the health-care system and consumers with what could be very large numbers of false-positive results. This is in contrast to the interpretation of diagnostic results that are clearly related to a patient's phenotype or clinical condition in which a lower threshold for reporting is appropriate.

Indications for diagnostic testing

1. WGS/WES should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:
 - a. 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.
 - b. 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.
 - c. A patient presents with a likely genetic disorder, but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.
 - d. 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.
 - i. Prenatal diagnosis by genomic (i.e., next-generation whole-exome or whole-genome) sequencing has significant limitations. The current technology does not support short turnaround times, which are often expected in the prenatal setting. There are high rates of false positives, false negatives, and variants of unknown clinical significance. These can be expected to be significantly higher than seen when array CGH is used in prenatal diagnosis.

Pretest considerations

2. Pretest counseling should be done by a medical geneticist or an affiliated genetic counselor and should include a formal consent process.
3. Before initiating WGS/WES, participants should be counseled regarding the expected outcomes of testing, the likelihood and type of incidental results that could be generated, and what results will or will not be disclosed.

4. As part of the pretest counseling, a clear distinction should be made between clinical and research-based testing. In many cases, findings will include variants of unknown significance that might be the subject for research; in such instances a protocol approved by an institutional review board must be in place and appropriate prior informed consent obtained from the participant.

Clinical testing and results reporting

5. Each and every component of the laboratory test (sequence acquisition, bioinformatics filtering, results interpretation, and reporting) should be performed in a laboratory directed by a board-certified individual with appropriate and broad medical genetics and genomics training. There should be an active dialogue between the laboratory and the ordering physician.
6. Test results can include:
 - a. Gene variants known to be associated with a phenotype that are relevant to the patient's condition;
 - b. Gene variants not known to be specifically associated with a phenotype but found to have compelling genetic, biological, and pathological features that implicate them in a patient's underlying phenotype;
 - c. Gene variants known to be associated with a phenotype but not believed to be related to the condition that led to the testing ("secondary findings").
7. Synthesizing the complex results of WGS/WES in the context of an individual patient and family in light of the clinical condition requires understanding of the testing and bioinformatic methodology, skills in pedigree analysis and interpretation, and familiarity with a broad range of inherited disorders (such as cancer predisposition, neurodegeneration, metabolic disorders, malformations, etc.). Synthesis and relevant medical decision making should be carried out by a qualified clinical geneticist working directly with the patient and family.
8. Laboratories and clinics using WGS/WES should have clear policies in place related to disclosure of secondary findings. Patients should be informed of those policies and the types of secondary findings that will be reported back to them and under what circumstances. Patients should be given the option of *not* receiving certain or secondary findings. Although these policies should be in place, exceptional circumstances will undoubtedly arise that need to be handled judiciously on a case-by-case basis through consultation between the ordering physician and the laboratory director.
9. Clinical laboratories should be strongly encouraged to share genotypic data from WGS/WES in public databases in order to more rapidly generate information that will lead to improved care.

Genetic screening

All of the aforementioned recommendations apply to the application of WGS/WES for diagnostic purposes. These technologies

will also likely be employed in the absence of specific clinical indications for testing, such as for a variety of screening purposes. In these contexts:

10. WGS/WES may be considered in preconception carrier screening, using a strategy to focus on genetic variants known to be associated with significant phenotypes in homozygous or hemizygous progeny. In view of the long turnaround times and interpretive complexities currently associated with this technology, preconception carrier screening is strongly favored over postconception screening.
11. WGS/WES should not be used at this time as an approach to prenatal screening.
12. WGS/WES should not be used as a first-tier approach for newborn screening.

13. Asymptomatic individuals interested in WGS/WES for purposes of health screening should receive both pretest and posttest counseling from a trained medical geneticist and/or affiliated genetic counselor. They should be informed of the potential risks and benefits of such testing and the virtual certainty of finding variants of uncertain significance. The threshold for determining which results should be returned to individuals seeking screening should be set significantly higher than that set for diagnostic testing due to the much lower a priori chance of disease in such individuals.

Posttest considerations

14. Genetic services and other appropriate specialist interventions associated with clinically relevant results should be available and accessible to those tested.