REVIEW ARTICLE

FRONTIERS IN MEDICINE

Next-Generation Sequencing to Diagnose Suspected Genetic Disorders

David R. Adams, M.D., Ph.D., and Christine M. Eng, M.D.

Clinical next-generation sequencing is being used frequently in medical practices in which genetic testing has traditionally taken place — for example, medical genetics and medical subspecialties such as neurogenetics. Emerging diagnostic applications include rapid-reporting approaches in intensive care settings (especially neonatal and pediatric)[1] and use early in the course of complex disease.[2] Large-scale projects in the United States, China, and elsewhere are exploring and developing the role of clinical next-generation sequencing in precision medicine.[3,4] This suggests a future in which genomic data will influence medical decision making for a diverse and growing group of patients (see video).

From the Office of the Clinical Director, National Human Genome Research Institute, and the Undiagnosed Diseases Program, National Institutes of Health, Bethesda, MD (D.R.A.); and the Department of Molecular and Human Genetics, Baylor College of Medicine, and Baylor Genetics — both in Houston (C.M.E.). Address reprint requests to Dr. Adams at the Undiagnosed Diseases Program, National Institutes of Health, Bldg. 10, Rm. 10C103E, 10 Center Dr., Bethesda, MD 20892, or at david.adams@nih.gov.

N Engl J Med 2018;379:1353-62.
DOI: 10.1056/NEJMra1711801

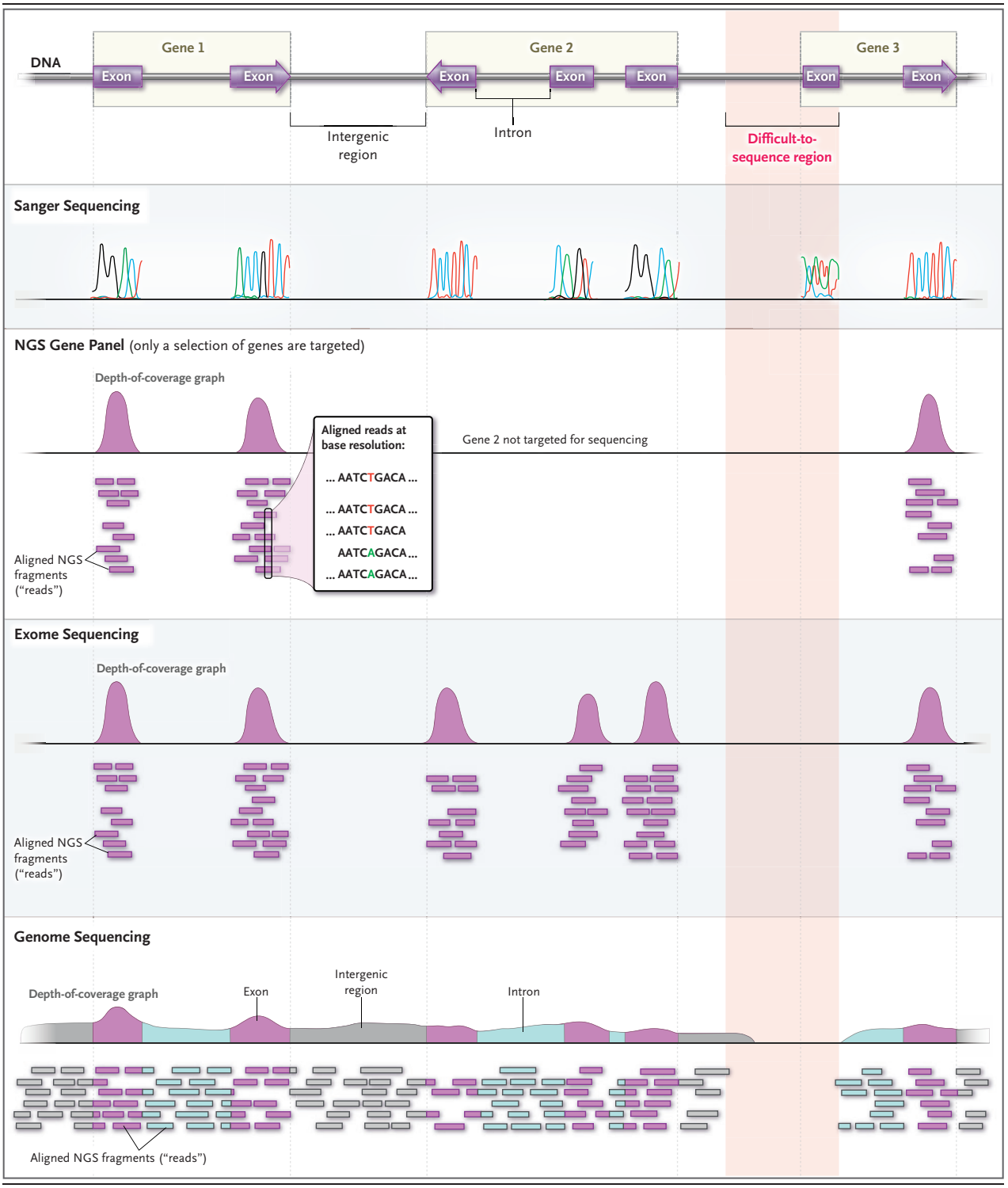
An illustrated glossary and a video overview of next-generation sequencing are available at NEJM.org

Clinical Next-Generation Sequencing as a Diagnostic Tool

The laboratory techniques that are used in clinical next-generation sequencing have been described in numerous reviews[5]; proposed guidelines for their application to diagnostic testing have been published.[6] The technology generates accurate and reliable sequence information for most parts of the genome. In a comparison of data from exome sequencing and Sanger sequencing (considered the standard of sequencing) for 684 participants in five genes, the validation rate for the exome sequencing results was 99.97%. Furthermore, discrepant results in high-quality exome sequencing regions were more likely to be correct in the exome sequencing data than in the first round of Sanger sequencing data.[7]

A clinical next-generation sequencing test can be designed to target a panel of selected genes, the exome (all known genes, or approximately 1 to 2% of the genome), or the entire genome. Gene panels target curated sets of genes associated with specific clinical phenotypes. Phenotypes may be narrow, with 4 genes in the panel for familial hypercholesterolemia, or broad, with more than 1000 genes in the panel for intellectual disability. Clinical exome sequencing targets approximately 22,000 protein-coding genes. Clinical genome sequencing is untargeted, generating sequence data from a region that is 50 to 100 times as large as that covered by exome sequencing and that includes regulatory, intronic, and intergenic regions (Fig. 1).

Clinical decision making about which test to order is an area of active research. Genome sequencing generates more uniform sequencing in some regions than does exome sequencing. Emerging analytic approaches can use genome sequencing to detect structural variants and expansion of short nucleotide repeats associated with disease. However, bioinformatic tools for genome sequencing are overall less developed than those available for exome sequencing. In addition, the cost of genome sequencing remains higher than that of exome sequencing, partly because of the cost of data management and analysis.



The primary goal for any diagnostic genetic test is the identification of DNA sequence variants that may be confidently associated with the presenting signs and symptoms. Other test results may identify potential risk variants for genetic disease that is absent or has not been diagnosed at the time of testing; these results are referred to as secondary, incidental, or medically actionable findings. Pa-

Figure 1 (facing page). Clinical Next-Generation Sequencing (NGS) Test Types.

Exome, genome, and panel NGS tests have different genomic coverage characteristics. NGS gene panel tests cover a set of genes defined by the clinical diagnostic laboratory. The panel will typically cover genes associated with a set of related medical conditions (e.g., heritable epilepsy disorders). Exome sequencing covers the majority of known genes, including genes that have not yet been associated with human disease. Genome sequencing covers a majority of both genes and intergenic regions. Each test type has an associated pattern of false negative results. For instance, a gene panel may not include a mutated gene and an exome may miss deep intronic splice mutation. In addition, some regions of the genome are difficult to sequence with any existing method.

tients with these risk variants may benefit from early screening and management efforts. Guidelines for the clinical reporting of this category of findings have been published.[8]

VARIANT CLASSIFICATION

Next-generation sequencing generates thousands of sequence variants that must be filtered and prioritized for clinical interpretation, which results in the reporting of a limited number of variants per report. This process may differ slightly among individual laboratories, but it generally includes annotation of variants, application of frequency filters and database searches to enrich for rare variants and eliminate common variants, and prediction of functional effect. Clinical evaluation of a DNA sequence variant includes an assessment of potential effects on the function of one or more genes and an assessment of the evidence supporting attribution of the illness at presentation to the affected gene or genes.[9] Both assessments benefit from strong association information (e.g., variant to disease and absence of variant to absence of disease).[10] However, such evidence may be difficult to obtain for rare variants or diseases.

Variants are evaluated according to evolutionary conservation, population frequency, and modeled (or measured) effect on protein function. Large-scale genomic sequencing databases, including the Genome Aggregation Database (gnomAD), are powerful tools for distinguishing common and rare variants.[11] Variant evaluation criteria have been published,[12] with subsequent proposed refinements.[13] These criteria include widely used assessment categories: pathogenic, likely pathogenic, likely benign, benign, and variant of unknown significance. Databases of previously assessed variants, such as ClinVar, have been established to collect and distribute information about previously interpreted variants.[14] ClinVar uses a categorical rating system to indicate the level of evidence for submitted interpretations. Variants are also prioritized on the basis of association with the phenotype of the patient, although the possibility of phenotypic heterogeneity and blended phenotypes (more than one mendelian disorder manifesting in an individual patient) must be considered.[15]

Clinical laboratories primarily report variants in genes for which the gene–disease association is well established. In other cases, the proposed association will be novel, creating an "N=1" situation (in which the diagnosis cannot be claimed to be definitive) and the opportunity to establish a new gene–disease association.[10] The risk of falsely associating diseases with genes and variants is regularly illustrated by the reclassification of previously established pathogenic variants as the result of improvements in frequency databases.[16] One innovative way to locate additional cases is through the use of matching databases. GeneMatcher (https://genematcher.org/), DECIPHER (https://decipher.sanger.ac.uk/), and PhenomeCentral (https://www.phenomecentral.org/) identify matching cases with the use of deidentified data, such as gene names or disease features.[17-19] The Matchmaker Exchange protocol allows matches between such databases.[20] These tools are publicly available and do not require computational expertise.

DIAGNOSTIC RATE AND TESTING STRATEGY

GENE PANELS

Gene panels (selected genes sequenced by a next-generation sequencing method) often have higher diagnostic rates than exome sequencing or genome sequencing, being designed to maximize coverage, sensitivity, and specificity for the included genes. An exception may occur in the context of greater diagnostic uncertainty, for which modeled data suggest that exome sequencing can have a higher diagnostic rate.[21] For example, in a study involving 50 patients with peripheral neuropathy, a virtual panel was derived from a subset of exome sequencing data. With the use of this panel, 11 of

50 diagnoses were made successfully. A subsequent analysis with the full set of exome data yielded 8 additional diagnoses.[22]

Panels are often used in the context of a specific suspected disease or group of diseases. Diagnostic rates vary among gene panels. For example, a 222-gene panel designed for inherited retinal diseases yielded a diagnosis in 98 of 192 patients (51%) with inherited retinal disorders.[23] A more genetically heterogeneous phenotype, early-onset epilepsy, had a diagnostic rate of approximately 30% with the use of a targeted panel of 172 genes associated with the phenotype.[24] A total of 156 of the 172 genes in the panel showed no abnormalities, which highlights the fact that the diagnostic rate may not increase linearly with the number of included genes.[25]

The cost of next-generation sequencing gene panels is variable but is often lower than that of exome sequencing. More expensive panels may incorporate other sequencing techniques to improve the reliability of detection of nucleotide repeat mutations or add procedures for detecting deletions and duplications.

CLINICAL GENOME AND EXOME SEQUENCING

Clinical genome and exome sequencing is often used for patients with previous negative panel studies or complex phenotypes for which the differential diagnosis is broad. These approaches have the benefit of assessing all known disease genes, while simultaneously providing a substrate for future reanalysis as variant classification and new gene discovery proceed. When clinical genome and exome sequencing is used in a patient with a suspected genetic disorder but without a diagnosis, the rate at which testing reveals a molecular diagnosis that is probably explanatory ranges from 25 to 52%.[26-29] Depending on the indication, this diagnostic rate may exceed that of other widely used genetic diagnostic tools such as chromosome microarray analysis.[30]

An improvement in diagnostic rates, in one example by 16 percentage points,[31] has been reported when sequencing in the affected person (proband) is performed concurrently with sequencing in the biologic parents (trio testing). This approach highlights the importance of communicating the clinical phenotype of all tested persons to the testing laboratory. With accurate information, a new (de novo) mutation in the proband can be confirmed to be absent from the unaffected parents, and an expected pattern of segregation can be confirmed for recessive diseases; this kind of information strengthens confidence in the diagnosis.

The actual diagnostic rate is highly dependent on the tested population, the availability of additional family members, and the definition of a high-likelihood diagnosis; rates of up to 60% have been reported in selected disease cohorts.[32] Diagnostic sensitivity may differ according to the affected organ system.[26] The remaining unexplained cases suggest that new genetic disorders are yet to be discovered and characterized. Potential biologic mechanisms for these disorders may include new mendelian disorders, gene interactions, epistasis, epigenetic mechanisms, uncaptured genetic variation (such as copy-number variation), and environmental contributions. Final clinical decisions about the appropriate testing strategy to use in a given context requires the incorporation of information about diagnostic uncertainty, panel design, cost, and the nature of any predictable disease-causing mutations (Table 1).

IMPLEMENTATION OF CLINICAL NEXT-GENERATION SEQUENCING

Clinicians who routinely use clinical next-generation sequencing have developed infrastructures for obtaining consent from patients and counseling them and their family members before and after testing. Testing begins with sample collection — typically, a blood sample (saliva, buccal swab, or blood spot may be acceptable, depending on the laboratory used) — and proceeds through a complex laboratory and analytic workflow (Fig. 2). Final reports include DNA sequence variants in genes known to be associated with the presenting illness, along with an assessment of potentially pathogenic variants (including those of uncertain significance). Variants in genes that are not associated with the presenting illness may also be included, such as predicted pathogenic variants in novel genes not currently associated with a specific disease.

There may be differences in reporting practices that may be specific to the particular test or diagnostic laboratory; thus, careful review of test characteristics and limitations is important for the ordering physician. A mock example report is shown in the Supplementary Appendix, available

Table 1. Sequence-Based Testing Formats.

Test Type	Design	Coverage	Usual Indication	Findings Unrelated to Presenting Illness or Clinical Question
Sanger-based sequencing of selected exons or mutations	Includes frequently encountered mutations or mutated exons	Well-defined*	Molecular confirmation of clinical diagnosis, hot-spot analysis, and analysis of known familial variants	Rare, but can occur for pleiotropic genes
Sanger-based full gene sequencing	All coding regions of gene, plus selected noncoding regions	Well-defined*	Molecular confirmation of clinical diagnosis	Rare, but can occur for pleiotropic genes
Sanger-based gene panel	Includes multiple disease-associated genes	Well-defined*	Clinical diagnosis of suspected condition with genetic heterogeneity	Rare, but can occur for pleiotropic genes
Next-generation sequencing gene panel	Variable†	Variably defined‡	Clinical diagnosis of suspected condition with genetic heterogeneity	Uncommon, but depends on size and design of panel
Exome sequencing	Near-complete sequencing of exons of known genes (1–2% of genome)	Partially defined§	Suspected genetic condition with broad differential diagnosis	Approximately 5% of studies, varies with reporting practices¶
Genome sequencing	Near-complete sequencing of genome	Partially defined§	Suspected genetic condition with broad differential diagnosis	Approximately 5% of studies, varies with reporting practices¶

* False negatives are rare, and the test result documentation includes a clear description of what is covered.
† There are two major approaches. In the first, exome-like capture technology is used, but only a subset of genes is captured. In the second, full exome data are collected, but only a subset of the genes is returned to the analyst.
‡ The laboratory may indicate that some regions may be missed by the sequencing technology. In many cases, regions that are not well sequenced by next-generation sequencing will be assessed with Sanger sequencing to avoid false negative results.
§ A minority of coding regions will not be sequenced by next-generation sequencing. Laboratories do not define these missed regions precisely.
¶ Variable practices include degree of adherence to American College of Medical Genetics and Genomics guidelines, return of carrier-status results, and other factors. Patients may be given choices in this regard during the consenting process.

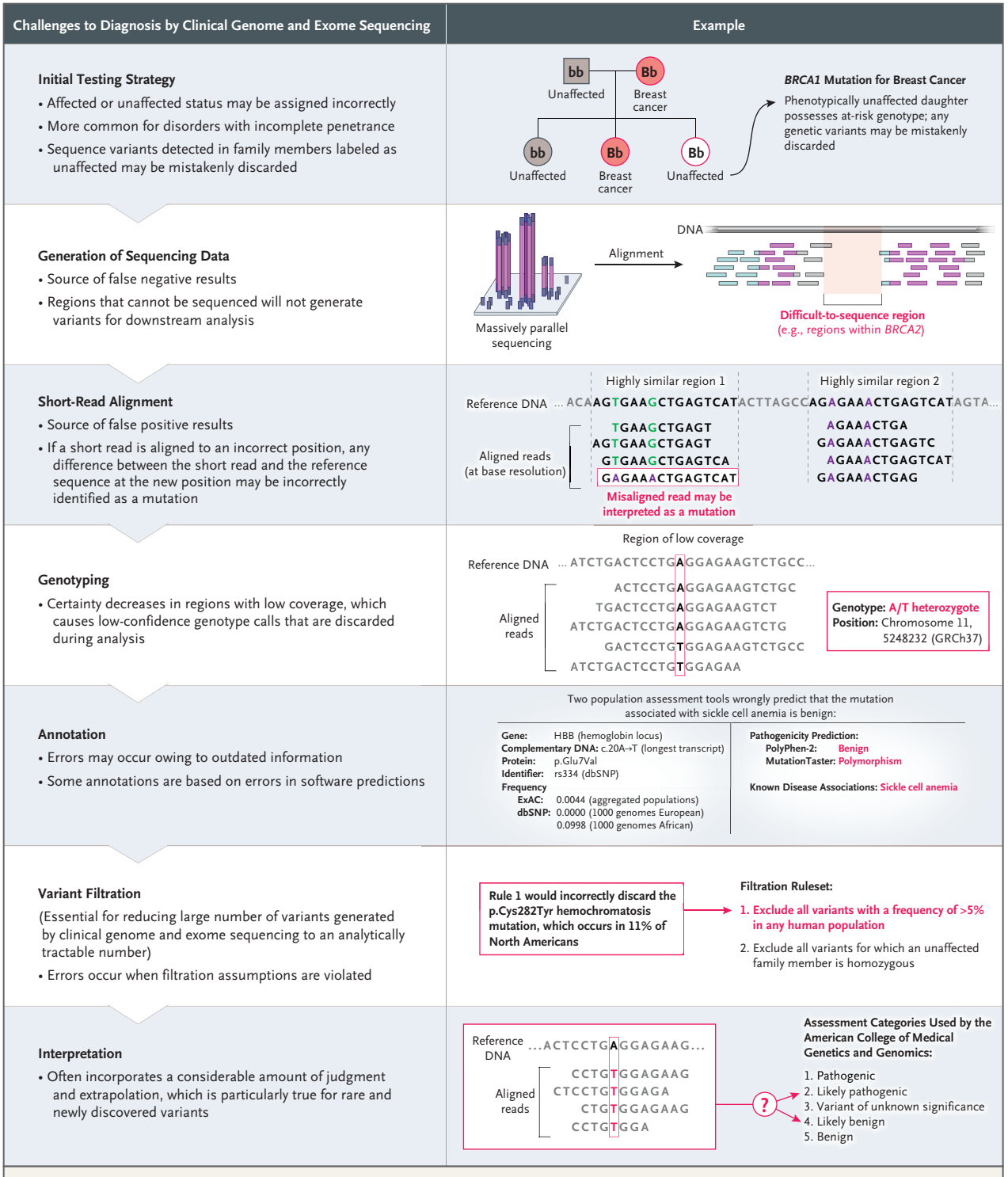


Figure 2. Laboratory and Analytic Workflow of Clinical NGS.

The term dbSNP denotes Database of Single-Nucleotide Polymorphisms, ExAC Exome Aggregation Consortium, and PolyPhen-2 Polymorphism Phenotyping, version 2.

with the full text of this article at NEJM.org. Most commercial testing laboratories have some means by which individual patients can request the release of their raw test data for reanalysis, second opinion, or research study.

Genetic results can provide support for clinical diagnoses, modify future disease risk, and inform the customization of a variety of therapies. Ongoing studies that incorporate results obtained by next-generation sequencing into point-of-care clinical practice may serve to illuminate the challenges of future widespread use of such sequencing, including the patient's right to decline receipt of certain types of results.[33] Informed consent is an important component of testing by clinical next-generation sequencing. A proper consenting process gathers information about secondary results that the patient would like to receive (if any) and provides counseling about the possibility of unanticipated risk variants being found. The American College of Medical Genetics and Genomics (ACMGG) has published a list of genes with both a clinically significant health association and a potential to modify therapeutic decision making.[8] Most current laboratories use this as a minimum set of secondary-result offerings. The consideration and return of other results, such as carrier status for recessive diseases, risk-modifying variants, and pharmacogenomic variants, are less standardized.

The consent process should also address potential risks of genetic testing, such as privacy and discrimination concerns. The Genetic Information Act of 2008 prohibits genetic discrimination in employment and health insurance, but the ability to obtain life, disability, or long-term care insurance is not protected against genetic discrimination.[34]

REIMBURSEMENT

Coverage of the cost of clinical next-generation sequencing (and analysis of the results) by both public and private payers lags behind the technological advances that have brought next-generation sequencing into clinical use.[35] Payers often consider several factors when making coverage decisions. These include the analytic and clinical validity of the test, guidelines from professional societies, and evidence-based scientific literature. Coverage decisions are generally based on whether the use of the test in clinical practice is considered to be experimental, investigational, or medically necessary.

Reimbursement for diagnostic testing by means of next-generation sequencing gene panels, exome sequencing, and genome sequencing may differ according to carrier and specific plan. Preauthorization by the payer is typically required. The ordering physician must provide clinical notes justifying the testing, including details of how medical management will be affected by the test results. Appeals of claim denials and peer-to-peer discussions with a payer medical director can be expected. Appeals of claim denials may incorporate diagnostic rates and other data obtainable from the clinical diagnostic laboratory. Self-pay options and financial-assistance plans offered by some testing laboratories can help improve access when coverage is denied. In addition, there has been an overall decline in the cost of genomic sequencing in recent years. Ultimately, studies of clinical usefulness and cost-effectiveness will be needed to improve coverage and access for patients and families.

STUDIES OF CLINICAL USEFULNESS

Timely diagnoses can alter medical management, provide accurate information about recurrence risk for family planning, and may result in health care savings by ending diagnostic odysseys. In a study involving 44 children who were selected by clinical geneticists, a diagnosis was achieved in 23 (52%) by proband-only exome sequencing. Clinical management was altered in 25%. The mean time to diagnosis was 6 years, with the incurring of costs that would have been saved had exome sequencing been carried out earlier.[27]

In another study, exome sequencing in 63 critically ill infants yielded a diagnostic rate of 51% at a mean age of 33.1 days of life and had an effect on medical management in 72%.[1] In the same study, 39 of 81 deceased infants received a diagnosis by exome sequencing.

A study in which genome sequencing was compared with a standard battery of genetic tests in 42 patients showed diagnostic yields of 43% and 10%, respectively.[36] Clinical usefulness was shown in 31%, and the estimated savings due to

changes in management approached $1 to 2 million total for a group of 6 patients. The performance of genome sequencing in patients for whom exome sequencing is nondiagnostic has been reported to show some additional usefulness.[37]

CHALLENGES AND OPPORTUNITIES

Clinical next-generation sequencing technology has evolved rapidly, frequently outpacing available resources for generating standards, guidelines, and resources. Examples include the storage of genomic data in electronic medical records (EMRs), data reanalysis, and the creation of databases of genomic variation in global populations.

Storage practices for genomic data in EMRs are heterogeneous. Models for integration into the EMR for ongoing patient care are being studied, such as in the Electronic Medical Records and Genomics (eMERGE) and Implementing Genomics in Practice (IGNITE) networks.[38]

A clinical sequencing report is usually prepared with the best evidence available at the time. As new information accrues, reanalysis of the test data may result in the reclassification of DNA variants of previously unclear clinical significance. In a recent study, exome reanalysis 12 months after the initial interpretation yielded additional diagnoses and was found to be a cost-effective diagnostic approach.[39] For the ordering physician, reanalysis may continue to produce new results over time but has its own risks and benefits, including loss of contact with patients for whom new results become available.

HEALTHY PERSONS

Genomic data are a potential component of precision medicine, and exome and genome sequences have been described as a lifelong clinical resource.[40,41] In addition to the uses described above, these data can potentially produce refinement of risk estimates for common diseases, pharmacogenomic data, and diagnoses for late-onset disorders. Exome-sequencing studies detect one to seven carrier variants on average, and one trial[42] showed that 2% of studies produce potentially actionable pathogenic or likely pathogenic variants in at least one of the genes recommended by the ACMGG for mandatory secondary-result reporting.[8] Approximately 130 pharmacogenomic biomarkers are included in current drug labeling,[43] but the literature regarding the usefulness of pharmacogenomic data for individual variants has been mixed. Most persons have one or more such variants, with an eMERGE Network study showing a median of two per person in a 5000-person cohort.[44] However, studies of genotype-guided warfarin dosing — arguably one of the best-known pharmacogenomic examples — have not yielded clear guidance.[45]

Assessment of common-disease risk and other uses of genomic data in healthy persons needs to be performed with the use of high-quality scientific methods despite the temptation to move rapidly toward implementation.[46] In a study of genome sequence analysis involving healthy patients, 22% had a monogenic disease risk result with uncertain clinical usefulness.[47] Ongoing and future studies are needed to expand characterization of genomic variation in diverse populations. A mismatch between the ancestry or ethnic group of the tested person and that of the available population ("control") data can negatively affect test performance.

FUTURE DIRECTIONS

The field of clinical genome and exome sequencing is evolving rapidly, with numerous projects focused on the expansion of diagnostic yield. Current areas of interest include the integration of RNA testing,[48] detection of structural variants,[49,50] and the improvement of decision making related to testing alternatives (gene panels, exomes, and genome testing).[51] Ongoing and planned genomics and health studies are adding to our understanding of the relationship between genomic variation and disease.[52,53] Future clinical initiatives that incorporate clinical next-generation sequencing into routine medical care are likely to herald a major increase in the total number of existing human genome and exome sequences. Cost, ethics, and standards development will help to shape the trajectory of broader incorporation of clinical next-generation sequencing and related forms of technology into routine medical practice. Given the rapid pace of changes during the past 5 years, all medical providers should keep a weather eye open for changes in this transformative field.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

An audio interview with Dr. Adams is available at NEJM.org

We thank William Gahl, M.D., Ph.D., Thomas Markello, M.D., Ph.D., and Vito Oliveri, M.B.A., for their review of an earlier version of the manuscript, discussions, and contributions to earlier versions of the figures.

REFERENCES

1. Meng L, Pammi M, Saronwala A, et al. Use of exome sequencing for infants in intensive care units: ascertainment of severe single-gene disorders and effect on medical management. JAMA Pediatr 2017;171:e173438.
2. Stark Z, Schofield D, Alam K, et al. Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. Genet Med 2017;19:867-74.
3. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med 2015;372:793-5.
4. Cyranoski D. China embraces precision medicine on a massive scale. Nature 2016;529:9-10.
5. Priest JR. A primer to clinical genome sequencing. Curr Opin Pediatr 2017;29:513-9.
6. Bowdin S, Gilbert A, Bedoukian E, et al. Recommendations for the integration of genomics into clinical practice. Genet Med 2016;18:1075-84.
7. Beck TF, Mullikin JC. Systematic evaluation of Sanger validation of next-generation sequencing variants. Clin Chem 2016;62:647-54.
8. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med 2017;19:249-55.
9. Richards CS, Bale S, Bellissimo DB, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. Genet Med 2008;10:294-300.
10. MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. Nature 2014;508:469-76.
11. Karczewski KJ, Weisburd B, Thomas B, et al. The ExAC browser: displaying reference data information from over 60 000 exomes. Nucleic Acids Res 2017;45:D1:D840-D845.
12. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
13. Biesecker LG, Harrison SM. The ACMG/AMP reputable source criteria for the interpretation of sequence variants. Genet Med 2018 March 15 (Epub ahead of print).
14. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018;46:D1:D1062-D1067.
15. Posey JE, Harel T, Liu P, et al. Resolution of disease phenotypes resulting from multilocus genomic variation. N Engl J Med 2017;376:21-31.
16. Shah N, Hou YC, Yu HC, et al. Identification of misclassified ClinVar variants via disease population prevalence. Am J Hum Genet 2018;102:609-19.
17. Buske OJ, Girdea M, Dumitriu S, et al. PhenomeCentral: a portal for phenotypic and genotypic matchmaking of patients with rare genetic diseases. Hum Mutat 2015;36:931-40.
18. Firth HV, Richards SM, Bevan AP, et al. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet 2009;84:524-33.
19. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat 2015;36:928-30.
20. Sobreira NLM, Arachchi H, Buske OJ, et al. Matchmaker Exchange. Curr Protoc Hum Genet 2017;95:9.31.1-9.31.15.
21. Dillon OJ, Lunke S, Stark Z, et al. Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. Eur J Hum Genet 2018;26:644-51.
22. Walsh M, Bell KM, Chong B, et al. Diagnostic and cost utility of whole exome sequencing in peripheral neuropathy. Ann Clin Transl Neurol 2017;4:318-25.
23. Consugar MB, Navarro-Gomez D, Place EM, et al. Panel-based genetic diagnostic testing for inherited eye diseases is highly accurate and reproducible, and more sensitive for variant detection, than exome sequencing. Genet Med 2015;17:253-61.
24. Rim JH, Kim SH, Hwang IS, et al. Efficient strategy for the molecular diagnosis of intractable early-onset epilepsy using targeted gene sequencing. BMC Med Genomics 2018;11:6.
25. Kreek MJ. Goals and rationale for pharmacotherapeutic approach in treating cocaine dependence: insights from basic and clinical research. NIDA Res Monogr 1997;175:5-35.
26. Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. Genet Med 2016;18:696-704.
27. Tan TY, Dillon OJ, Stark Z, et al. Diagnostic impact and cost-effectiveness of whole-exome sequencing for ambulant children with suspected monogenic conditions. JAMA Pediatr 2017;171:855-62.
28. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med 2013;369:1502-11.
29. Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA 2014;312:1870-9.
30. Lionel AC, Costain G, Monfared N, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. Genet Med 2018;20:435-43.
31. Farwell KD, Shahmirzadi L, El-Khechen D, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med 2015;17:578-86.
32. Ghaoui R, Cooper ST, Lek M, et al. Use of whole-exome sequencing for diagnosis of limb-girdle muscular dystrophy: outcomes and lessons learned. JAMA Neurol 2015;72:1424-32.
33. Jarvik GP, Amendola LM, Berg JS, et al. Return of genomic results to research participants: the floor, the ceiling, and the choices in between. Am J Hum Genet 2014;94:818-26.
34. Genetic Information Nondiscrimination Act of 2008. Washington, DC: Equal Employment Opportunity Commission (https://www.eeoc.gov/laws/statutes/gina.cfm).
35. Deverka PA, Kaufman D, McGuire AL. Overcoming the reimbursement barriers for clinical sequencing. JAMA 2014;312:1857-8.
36. Farnaes L, Hildreth A, Sweeney NM, et al. Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization. NPJ Genom Med 2018;3:10.
37. Alfares A, Aloraini T, Subaie LA, et al. Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. Genet Med 2018 March 22 (Epub ahead of print).
38. Weitzel KW, Alexander M, Bernhardt BA, et al. The IGNITE network: a model for genomic medicine implementation and research. BMC Med Genomics 2016;9:1.
39. Ewans LJ, Schofield D, Shrestha R, et al. Whole-exome sequencing reanalysis at 12 months boosts diagnosis and is cost-effective when applied early in Mendelian disorders. Genet Med 2018 March 29 (Epub ahead of print).

40. Biesecker LG. Opportunities and challenges for the integration of massively parallel genomic sequencing into clinical practice: lessons from the ClinSeq project. Genet Med 2012;14:393-8.
41. Lindor NM, Thibodeau SN, Burke W. Whole-genome sequencing in healthy people. Mayo Clin Proc 2017;92:159-72.
42. Biesecker BB, Lewis KL, Umstead KL, et al. Web platform vs in-person genetic counselor for return of carrier results from exome sequencing: a randomized clinical trial. JAMA Intern Med 2018;178:338-46.
43. Table of pharmacogenomic biomarkers in drug labeling. Silver Spring, MD: Food and Drug Administration, 2017 (https://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm).
44. Bush WS, Crosslin DR, Owusu-Obeng A, et al. Genetic variation among 82 pharmacogenes: the PGRNseq data from the eMERGE Network. Clin Pharmacol Ther 2016;100:160-9.
45. Tavares LC, Marcatto LR, Santos PC. Genotype-guided warfarin therapy: current status. Pharmacogenomics 2018;19:667-85.
46. Ioannidis JPA, Khoury MJ. Evidence-based medicine and big genomic data. Hum Mol Genet 2018;27:R1:R2-R7.
47. Vassy JL, Christensen KD, Schonman EF, et al. The impact of whole-genome sequencing on the primary care and outcomes of healthy adult patients: a pilot randomized trial. Ann Intern Med 2017 June 27 (Epub ahead of print).
48. Volk AE, Kubisch C. The rapid evolution of molecular genetic diagnostics in neuromuscular diseases. Curr Opin Neurol 2017;30:523-8.
49. Zepeda-Mendoza CJ, Ibn-Salem J, Kammin T, et al. Computational prediction of position effects of apparently balanced human chromosomal rearrangements. Am J Hum Genet 2017;101:206-17.
50. Barseghyan H, Tang W, Wang RT, et al. Next-generation mapping: a novel approach for detection of pathogenic structural variants with a potential utility in clinical diagnosis. Genome Med 2017;9:90.
51. Kuo FC, Mar BG, Lindsley RC, Lindeman NI. The relative utilities of genome-wide, gene panel, and individual gene sequencing in clinical practice. Blood 2017;130:433-9.
52. The 100,000 Genomes Project. London: Genomics England (https://www.genomicsengland.co.uk/the-100000-genomes-project/).
53. All of Us research program. Bethesda, MD: National Institutes of Health, 2017 (https://allofus.nih.gov/).