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) and use early in the course of complex disease. Large-scale projects in the United States, China, and elsewhere are exploring and developing the role of clinical next-generation sequencing in precision medicine. This suggests a future in which genomic data will influence medical decision making for a diverse and growing group of patients (see video).

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; proposed guidelines for their application to diagnostic testing have been published. 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.

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-
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. Both assessments benefit from strong association information (e.g., variant to disease and absence of variant to absence of disease). However, such evidence may be difficult to obtain for rare variants or diseases.

Variants are evaluated according to evolutionary conservatism, 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. Variant evaluation criteria have been published, with subsequent proposed refinements. 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. 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.

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. 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. 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. The Matchmaker Exchange protocol allows matches between such databases. 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. 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.

\textbf{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%.\textsuperscript{26-29} Depending on the indication, this diagnostic rate may exceed that of other widely used genetic diagnostic tools such as chromosome microarray analysis.\textsuperscript{30}

An improvement in diagnostic rates, in one example by 16 percentage points,\textsuperscript{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.\textsuperscript{32} Diagnostic sensitivity may differ according to the affected organ system.\textsuperscript{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).
<table>
<thead>
<tr>
<th>Test Type</th>
<th>Design</th>
<th>Coverage</th>
<th>Usual Indication</th>
<th>Findings Unrelated to Presenting Illness or Clinical Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanger-based sequencing of selected exons or mutations</td>
<td>Includes frequently encountered mutations or mutated exons</td>
<td>Well-defined*</td>
<td>Molecular confirmation of clinical diagnosis, hot-spot analysis, and analysis of known familial variants</td>
<td>Rare, but can occur for pleiotropic genes</td>
</tr>
<tr>
<td>Sanger-based full gene sequencing</td>
<td>All coding regions of gene, plus selected noncoding regions</td>
<td>Well-defined*</td>
<td>Molecular confirmation of clinical diagnosis</td>
<td>Rare, but can occur for pleiotropic genes</td>
</tr>
<tr>
<td>Sanger-based gene panel</td>
<td>Includes multiple disease-associated genes</td>
<td>Well-defined*</td>
<td>Clinical diagnosis of suspected condition with genetic heterogeneity</td>
<td>Rare, but can occur for pleiotropic genes</td>
</tr>
<tr>
<td>Next-generation sequencing gene panel</td>
<td>Variable†</td>
<td>Variably defined‡</td>
<td>Clinical diagnosis of suspected condition with genetic heterogeneity</td>
<td>Uncommon, but depends on size and design of panel</td>
</tr>
<tr>
<td>Exome sequencing</td>
<td>Near-complete sequencing of exons of known genes (1–2% of genome)</td>
<td>Partially defined§</td>
<td>Suspected genetic condition with broad differential diagnosis</td>
<td>Approximately 5% of studies, varies with reporting practices¶</td>
</tr>
<tr>
<td>Genome sequencing</td>
<td>Near-complete sequencing of genome</td>
<td>Partially defined§</td>
<td>Suspected genetic condition with broad differential diagnosis</td>
<td>Approximately 5% of studies, varies with reporting practices¶</td>
</tr>
</tbody>
</table>

* 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.
Challenges to Diagnosis by Clinical Genome and Exome Sequencing

**Initial Testing Strategy**
- Affected or unaffected status may be assigned incorrectly
- More common for disorders with incomplete penetrance
- Sequence variants detected in family members labeled as unaffected may be mistakenly discarded

**Generation of Sequencing Data**
- Source of false negative results
- Regions that cannot be sequenced will not generate variants for downstream analysis

**Short-Read Alignment**
- Source of false positive results
- If a short read is aligned to an incorrect position, any difference between the short read and the reference sequence at the new position may be incorrectly identified as a mutation

**Genotyping**
- Certainty decreases in regions with low coverage, which causes low-confidence genotype calls that are discarded during analysis

**Annotation**
- Errors may occur owing to outdated information
- Some annotations are based on errors in software predictions

**Variant Filtration**
(Essential for reducing large number of variants generated by clinical genome and exome sequencing to an analytically tractable number)
- Errors occur when filtration assumptions are violated

**Interpretation**
- Often incorporates a considerable amount of judgment and extrapolation, which is particularly true for rare and newly discovered variants

**Example**

**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.31 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 (ACMG) 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 Nondiscrimination 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

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 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

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.\textsuperscript{17}

**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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{42} showed that 2% of studies produce potentially actionable pathogenic or likely pathogenic variants in at least one of the genes recommended by the ACMG for mandatory secondary-result reporting.\textsuperscript{8} Approximately 130 pharmacogenomic biomarkers are included in current drug labeling,\textsuperscript{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.\textsuperscript{44} However, studies of genotype-guided warfarin dosing — arguably one of the best-known pharmacogenomic examples — have not yielded clear guidance.\textsuperscript{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.\textsuperscript{46} In a study of genome sequence analysis involving healthy patients, 22% had a monogenic disease risk result with uncertain clinical usefulness.\textsuperscript{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,\textsuperscript{48} detection of structural variants,\textsuperscript{49,50} and the improvement of decision making related to testing alternatives (gene panels, exomes, and genome testing).\textsuperscript{51} Ongoing and planned genomics and health studies are adding to our understanding of the relationship between genomic variation and disease.\textsuperscript{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.
NEXT-GENERATION SEQUENCING AND GENETIC DISORDERS

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