

REVIEW ARTICLE

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Diagnostic Clinical Genome and Exome Sequencing

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SEQUENCING OF THE GENOME OR EXOME FOR CLINICAL APPLICATIONS, hereafter referred to as clinical genome and exome sequencing (CGES), has now entered medical practice.¹ Several thousand CGES tests have already been ordered for patients, with the goal of establishing diagnoses for rare, clinically unrecognizable, or puzzling disorders that are suspected to be genetic in origin. We anticipate increases in the use of CGES, the key attribute of which — its breadth — distinguishes it from other forms of laboratory testing. The interrogation of variation in about 20,000 genes simultaneously can be a powerful and effective diagnostic method.²

CGES has been hailed as an important tool in the implementation of predictive and individualized medicine, and there is intense research interest in the clinical benefits and risks of sequencing for screening healthy persons³; however, current practice recommendations⁴ do not support the use of sequencing for this purpose, and for that reason we do not further address it here. We have also limited this overview of CGES to the analysis of germline sequence variants for diagnostic purposes and do not discuss the use of CGES to uncover somatic variants in cancer in order to individualize cancer therapy.

Clinicians should understand the diagnostic indications for CGES so that they can effectively deploy it in their practices. Because the success rate of CGES for the identification of a causative variant is approximately 25%,⁵ it is important to understand the basis of this testing and how to select the patients most likely to benefit from it. Here, we summarize the technologies underlying CGES and offer our insights into how clinicians should order such testing, interpret the results, and communicate the results to their patients (an interactive graphic giving an overview of the process is available with the full text of this article at NEJM.org).



An interactive graphic giving an overview of the steps in CGES is available at NEJM.org

TECHNICAL OVERVIEW AND LIMITATIONS OF CGES

Detailed technical descriptions of sequencing can be found elsewhere,⁶⁻⁹ and we provide a graphical summary of one method of CGES in Figures 1 and 2. Regardless of the specific technology that is used, the process begins with the extraction of DNA from white cells, after which the DNA is broken into short fragments, the sequences of which are determined with the use of one of various sequencing technologies. The sequencing instrument generates millions of short sequence reads, which are strings of data representing the order of the DNA nucleotides, or bases, in each fragment. These sequence reads are then aligned to specific positions in the human genome reference sequence (see Glossary) with the use of computers.¹⁰ Similarities and differences between the patient's sequence and the reference sequence are tabulated, and a computerized determination of the specific genotype (A, C, G, or T) at each position in the exome or genome is performed, resulting in an output file along

with information representing the number of sequence reads generated (depth of coverage) and the accuracy of the genotype at each position. The output file is computationally filtered in accordance with the clinical objective of the test and the preferences of the laboratory. Typically, the file is filtered for variants that are rare or have not previously been reported (because it is reasoned that a common variant cannot cause a rare disease), variants predicted to cause a loss or altered function of a gene, and variants previously reported to cause disease.^{10,11}

CGES is most useful for the detection of single-nucleotide substitutions and insertions or deletions of 8 to 10 nucleotides or smaller; it is less accurate for other types of genomic variation (Table 1). The yield of sequence reads is inherently uneven across the exome (or genome) — typical results provide adequate coverage of 85 to 95% of the targeted sequence. With exome sequencing, there is also variable coverage of flanking intronic regions, which may include disease-causing variants that affect the splicing of messenger RNA encoded by the gene (splice variants).

INDICATIONS FOR ORDERING CGES

CGES is currently indicated for the detection of rare variants in patients with a phenotype suspected to be due to a mendelian (single-gene) genetic disorder, after known single-gene candidates have been eliminated from consideration or when a multigene testing approach is prohibitively expensive. Patients can be of any age but are commonly children, since many genetic conditions are manifested in childhood; evaluations are performed because parents are searching for the cause or for information to guide management and treatment and desire accurate information regarding the risk of recurrence, as noted below.

The preparation for ordering CGES should include four key elements: gathering information on family history, systematically evaluating the patient's phenotype, searching medical literature and databases, and obtaining informed consent. A thorough family history should be obtained to assess whether there are similar or related phenotypes in other family members, as well as to evaluate and assess the inheritance pattern. The patient (and other apparently affected family members) should be evaluated for

Glossary
Exome sequencing: DNA sequencing that targets the exons of all genes in the genome. The exome makes up about 1% of the genome, primarily exons of genes that code for proteins. This type of sequencing is sometimes referred to as “whole-exome sequencing,” even though coverage of the exons is not 100%.
Exons: Segments of genes that are spliced together after gene transcription to form messenger RNA, which, in turn, is translated into protein.
Expressivity: Variation in the severity of a genetic disorder among persons with some features of the condition.
Filtering analysis: The process of excluding DNA variants from further consideration because of various attributes, with the use of bioinformatics and manual curation. For example, most filtering analyses exclude synonymous variants (DNA variants that are predicted not to change the amino acid sequence of a protein).
Genome sequencing: DNA sequencing that targets the entire genome. It is sometimes termed “genome shotgun sequencing” or “whole-genome sequencing,” even though coverage is not 100%.
Germline variant: A DNA sequence variant that was transmitted by means of a gamete (sperm or egg) or that was caused by a mutation in the zygote or at a very early stage of fetal development and is presumed to be present in all of a person's nucleated cells.
Human genome reference sequence: A reference sequence that provides a haploid mosaic of different DNA sequences from multiple donors, which is revised periodically and is not necessarily normal.
Penetrance: The likelihood that a person with a causative variant in a gene has any recognizable symptom, sign, or laboratory feature of the disease associated with that variant.
Sanger sequencing: A method of sequence determination, invented by Frederick Sanger, that uses dideoxy terminator nucleotide chemistry, with the reaction products separated by gel electrophoresis.
Variant: A difference in a DNA sequence in comparison with the normal reference sequence. A variant may be benign (sometimes referred to as a polymorphism) or pathogenic (sometimes referred to as a mutation).

other potentially relevant manifestations. For example, if the primary presentation is autism and CGES is being considered by a neurologist, the patient should also be carefully examined for respiratory, cardiac, renal, skin, and dysmorphic abnormalities. With a family history and a comprehensive phenotype in hand, a literature review or syndrome database search should be performed (Table 2) to determine whether the patient's presentation matches a rare but estab-

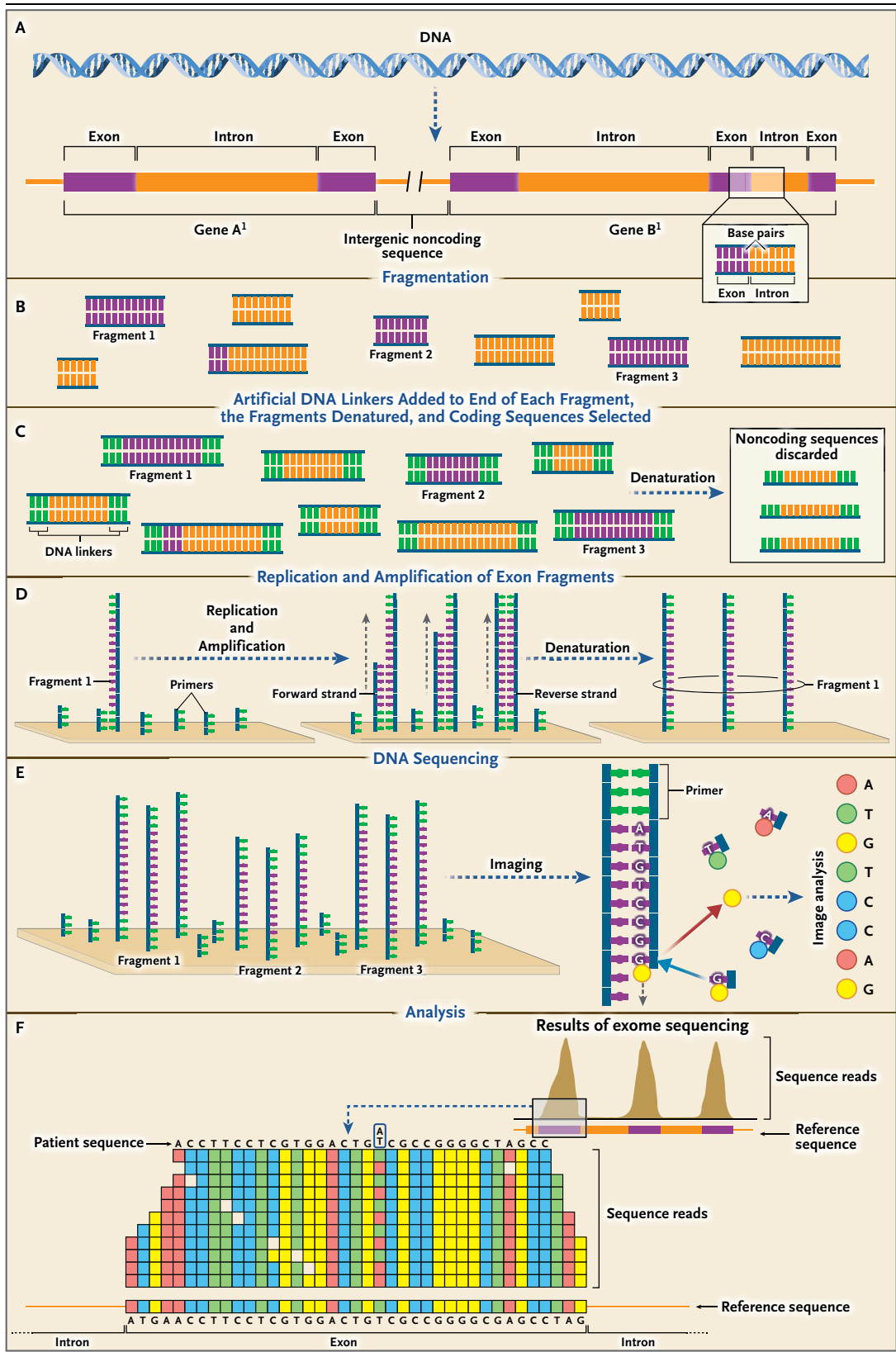


Figure 1 (facing page). Schematic Overview of Exome Sequencing.

Exome sequencing targets the approximately 1% of the genome that is made up of exons, which encode protein sequence. The DNA from the patient (Panel A) is isolated and broken into fragments (Panel B); the DNA fragments are coupled to artificial DNA linker segments (Panel C), and the fragments are selected with the use of artificial DNA or RNA baits that are complementary to targeted DNA (not shown). The sequencing process starts with the binding of the end of each DNA fragment to a solid matrix and in situ amplification (Panel D), and the DNA fragments are then sequenced on the slide in a series of reactions in which a complementary nucleotide with one of four colored fluorescent dyes is added to each cluster of identical molecules (Panel E). The identity of the colored fluorescent indicator of each cluster is imaged with a laser and a camera coupled to a microscope, the fluorescent indicator is removed, and the cycle is repeated to generate a nucleotide sequence read that is 75 to 150 nucleotides in length. The sequence reads are aligned to a reference DNA sequence (Panel F), and a genotype call for each position is made. In this example, most of the positions are homozygous reference sequence, but one position is called as heterozygous A/T. This figure illustrates one widely used sequencing technology, but it is not intended to endorse that technology over other methods.

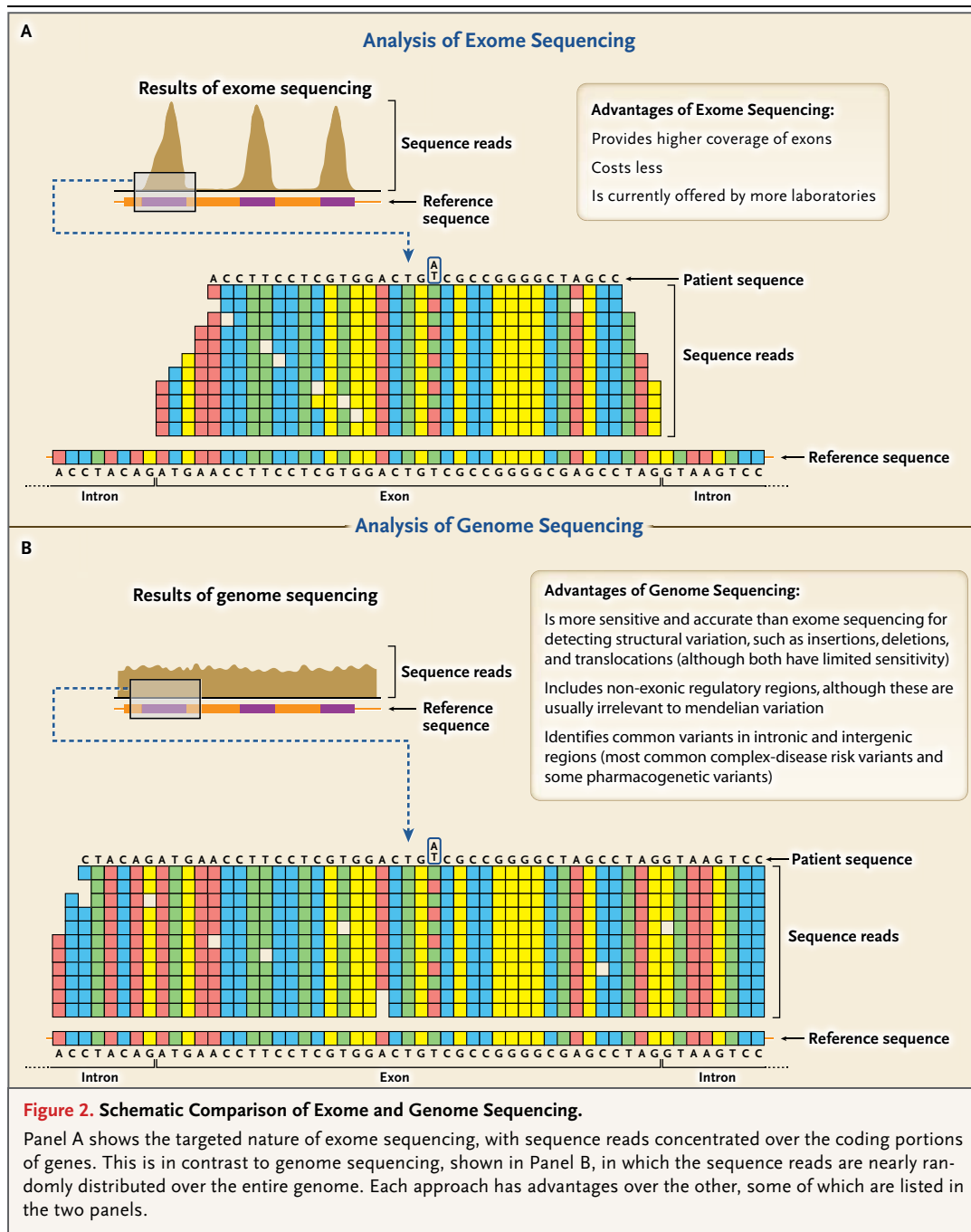


Table 1. DNA Variant Types Currently Not Well Detected or Undetectable by Clinical Genome and Exome Sequencing (CGES), with Examples of Phenotypes Associated with Such Variants.

Variant Type	Associated Phenotype or Phenotypes
Repetitive DNA, including trinucleotide repeats	Fragile X syndrome, Huntington's disease
Copy-number variants	DiGeorge syndrome (22q11.2 deletion syndrome), Charcot–Marie–Tooth disease type 1A
Long insertion–deletion variants*	Resistance to human immunodeficiency virus infection
Structural variants	Chromosomal translocations associated with spontaneous abortions
Aneuploidy	Down's syndrome, Turner's syndrome
Epigenetic alterations	Prader–Willi syndrome, Beckwith–Wiedemann syndrome

* There is a spectrum of genomic variants, from nucleotide insertions and deletions of at least 8 to 10 bp through copy-number variants, that are less effectively assayed by current CGES technology.

lished syndrome that could narrow the focus of genetic testing before CGES is undertaken. If CGES is performed, it may lead to the discovery of a known pathogenic variant or a previously undescribed and apparently pathogenic variant in a gene known to cause human disease. Some laboratories may also report novel, apparently pathogenic variants in genes not yet known to cause human disease. In such cases, additional clinical and laboratory research may be necessary to establish the pathogenicity of the variants.

The strategy for selecting family members to undergo the sequencing and analysis will be influenced by the expected inheritance patterns (e.g., dominant or recessive) and whether other family members with and without similar features are available for phenotyping and genetic testing. In some scenarios, it can be important to test unaffected relatives (Table 3). When a *de novo* variant is suspected, the relatives most commonly tested are the biologic parents of the patient; if neither parent has the variant and biologic parentage is confirmed, then the variant is indeed *de novo*.¹² When a recessively or dominantly inherited condition is suspected, parents, siblings, or even distant relatives may be tested, depending on the particular family history and with the understanding that if two family members are more distant, the number of potential candidate variants will be reduced. Thus, sequencing in two affected cousins will yield fewer candi-

date variants than sequencing in two affected siblings. In other situations, CGES performed in only the affected child, followed by genotyping of just a few variants in affected and unaffected relatives, may show cosegregation of the variant and the disease, which supports the pathogenicity of the variant. Some CGES laboratories can assist by offering advice about the testing strategy for a given clinical scenario, but this is no substitute for a robust understanding of these issues.

EVALUATING A CGES RESULT

The outcomes of CGES analysis vary widely. In some CGES reports, a single causative variant is asserted to be the likely cause of the disease, whereas in others, multiple candidate variants are identified that must then be evaluated by the ordering clinician or by consultants. In many cases, no plausible variants are identified.

The two main considerations for evaluating CGES results are their analytic validity and their clinical validity. Analytic validity is a measure of the likelihood that the patient actually has the particular genotype shown in the CGES results — that is, the accuracy of the test. Clinical validity, which is much more complicated and challenging to assess, is the determination that a particular disease is truly caused by variants in a particular gene and that the specific variant that has been detected is indeed pathogenic.

Positive CGES findings are highly accurate, but the false negative rate varies according to the genomic region. For this reason, CGES is not yet a substitute for targeted sequencing of suspected genes or gene panels that have been optimized for a particular condition.¹³ Most laboratories validate positive CGES results with well-established methods such as polymerase-chain-reaction amplification and Sanger sequencing. Confirmatory Sanger sequencing should be considered when any major medical intervention is being contemplated on the basis of a CGES result, if such confirmation is not routinely provided by the CGES laboratory.

As noted above, determining the clinical validity of CGES results is more challenging than determining their analytic validity. The general approach is to compare variants implicated by CGES with databases of known variation, which are in turn based on reports that describe causal variants as well as associations between variants

and phenotypes.¹⁴ In the literature, however, there are many false attributions of disease to variants, a problem that is in part due to the conflation of association with causation.¹⁵ Clinicians reviewing the results of sequencing should be aware of the possibility of a false attribution of pathogenicity to a variant¹⁶ and should realize that the chances of false attribution are increased in CGES because thousands of genes are tested simultaneously.

The clinical usefulness of identifying the variant that is the cause of a previously undiagnosed syndrome or heritable disorder varies. In some cases, it can lead to a specific treatment or management strategy that dramatically changes the clinical outcome.^{17,18} In the majority of cases in which the finding does not change clinical management, treatment, or prognosis, it may still be useful because it can end an expensive, potentially invasive, and stressful diagnostic odyssey. The identification of the causative variant may provide accurate estimates of recurrence risk and facilitate preconception intervention or prenatal diagnosis for the affected patient or affected or at-risk relatives. In adult-onset disease, one of the most useful outcomes of successfully identifying the causative variant is the subsequent detection of presymptomatic, at-risk siblings for whom screening or preventive therapy might improve the clinical outcome. Examples include enhanced surveillance or prophylactic surgery for patients found to have a genetic susceptibility to cancer.

Pretest counseling is particularly important, to maintain realistic expectations for finding the causative variant and to alert the patient or family that in most cases, a positive result is unlikely to change treatment or management decisions or to improve the prognosis.¹⁹ In addition, the patient should be advised that incidental findings unrelated to the reason for testing may be found and reported, as described below. It may also be important to discuss the cost of the test with the patient. As is the case with many medical services, assessment of the cost is complicated by many factors. The published billing charge for CGES in most laboratories is in the range of \$4,000 to \$15,000 per patient, with some laboratories offering lower per-person charges for family testing. To put this in perspective, the per-person charge for sequencing of an exome may be only two to four times the

Table 2. Examples of Online Databases to Assist Clinicians in Differential Diagnosis or Candidate-Gene Identification for Rare Syndromic Disorders before CGES Is Performed.

Free access (or an available free-access version)

Genetic Testing Registry (www.ncbi.nlm.nih.gov/gtr)
 HuGE Navigator (<http://hugenavigator.net/HuGENavigator>)
 Human Gene Mutation Database (www.biobase-international.com/product/hgmd)
 Online Mendelian Inheritance in Man (www.omim.org)
 Phenomizer (<http://compbio.charite.de/phenomizer>)
 SimulConsult (www.simulconsult.com)

Subscription or fee required for access

Isabel (www.isabelhealthcare.com/home/default)
 London Medical Databases (<http://lmdatabases.com>)
 POSSUM (www.possum.net.au)

published billing charge for some single-gene sequencing tests, which is why exome sequencing can be more efficient in a number of clinical scenarios. Some laboratories have reported that third-party payers are reimbursing for this testing, but practices vary widely, and patients should understand this in advance.

INTERPRETING AND COMMUNICATING CGES RESULTS

Clinicians should review the CGES results delivered by the laboratory geneticist and place the findings into context with other relevant medical considerations. Sometimes an identified variant will spur additional history taking or an additional examination of the patient, which may reveal clinical features of a previously unrecognized syndrome or lead to the conclusion that the variant is not related to the disorder in the patient (Table 4).

In some cases, the CGES report from the testing laboratory identifies a causative variant (or two variants for a recessive disorder) in a single gene that is considered sufficiently pathogenic and specific that a diagnostic association with a heritable disorder is strongly supported. Such a conclusion by the laboratory geneticist is typically based on the integration of the submitted clinical information with information on diseases associated with the identified variant.⁵ In this case, as with all laboratory tests, the order-

Table 3. Genetic Considerations in Deciding Which Relatives Should Undergo CGES.**Autosomal dominant inheritance suspected**

Testing is most valuable in the trio consisting of a child and both biologic parents if the disorder may be caused by a *de novo* variant of a gene associated with a disorder that is caused by heterozygous (monoallelic) mutations.

If a phenotype with autosomal dominant inheritance within a family is being evaluated, sequences from two distant relatives with the phenotype will be more valuable than sequences from two close relatives with the phenotype. Sequences from distant relatives are recommended because there will be fewer variants shared solely by chance in close relatives.

Autosomal recessive inheritance suspected

Testing can also be valuable in the trio of a child and both biologic parents if gene variants inherited in an autosomal recessive pattern are being considered.

If there is consanguinity, then testing the trio may be less helpful, because the child's sequence will already have large areas of homozygosity.

Table 4. Examples of How to Evaluate the Pathogenicity of a Variant.**Variant supported as pathogenic**

A CGES report for a boy with apparently isolated intellectual disability identified a novel, previously unidentified variant in the gene *ATRX*. This gene is associated with the α -thalassemia mental retardation syndrome. In response to this report, the clinician orders hematologic testing, which identifies a subtle thalassemia phenotype. This additional testing strongly supports the variant identified in the CGES result as the cause of the intellectual disability in the patient.

Variant not supported as pathogenic

A CGES report identified a variant in the gene *NF2* in a 2-month-old infant with congenital, bilateral sensorineural hearing loss. The variant is present in the Human Gene Mutation Database as a disease-causing variant associated with neurofibromatosis 2. However, that database entry is based on a single report²⁰ that did not specify whether the patient with the variant was a case patient, a patient with a suspected case, or a control. A review of neurofibromatosis 2 in GeneReviews (www.ncbi.nlm.nih.gov/books/NBK1201/) shows that this disorder typically causes unilateral hearing loss with an onset in young adulthood, not bilateral deafness with an onset in infancy. This post-hoc assessment — the absence of support in the literature for causality of the variant combined with data from GeneReviews on the clinical features known to be caused by mutations in *NF2* — suggests that the evidence for the pathogenicity of this variant is weak, and the variant is unlikely to explain the child's phenotype.

referral to a specialist, depending on the nature of the diagnosed disorder.

In other cases, the laboratory CGES report identifies one or more candidate variants that may or may not be the cause of the phenotype that triggered ordering of the test. Additional literature and database searching, additional phenotyping, and even functional studies may be needed to winnow these candidates down to the causative variant, if indeed it is among these candidates. This approach to post-test clinical evaluation²¹ requires a high level of expertise in genetics and informatics, as well as knowledge of the clinical features of the phenotype in the patient and of the phenotypes associated with the identified variants and genes. The details of this type of evaluation are beyond the scope of this review, but such evaluations may require consultation with geneticists or other disease-specific experts and the enrollment of patients and their families in studies.

If a CGES result is negative, subsequent improvements in knowledge may lead to the recognition that a previously uninterpretable variant in a negative CGES result is in fact pathogenic. The methods and approaches for ongoing reanalysis of CGES results have not been established, but it should eventually be possible to regularly reanalyze such results with the goal of identifying previously unknown variants. How this will be done and how it will be reimbursed are not yet known. These issues aside, counseling a family about a negative CGES result can be challenging, because the patient may be disappointed with an inconclusive outcome of such an extensive and expensive test. It is important to communicate to the patient or to the parents of a tested child that a negative result of CGES testing does not reliably rule out the presence of a causative variant.

INCIDENTAL FINDINGS

ing clinician should confirm that this conclusion is clinically appropriate before making the diagnosis by reviewing available data from trusted medical sources (e.g., PubMed, GeneReviews, or textbooks). The patient — and, with permission, the patient's family — should be provided with information on the inheritance, penetrance, expressivity, prognosis, and in some cases specific treatment or management for the condition, either by the ordering clinician or through

CGES can generate results unrelated to the indication that prompted sequencing, and these results may be clinically useful.^{19,22} As with all testing, it is important to constrain such evaluations to avoid unnecessary future testing and expense. To this end, the American College of Medical Genetics and Genomics has recommended that the laboratories providing CGES routinely seek and report to the ordering clinician specific variants in a minimum set of 56

genes representing 24 disorders that are highly medically actionable.²² It is estimated that 1 to 3% of patients undergoing CGES have such a finding,²³ which may be outside the expertise of the clinician who ordered the test. A clinician with expertise in the identified disorder should review the personal and family history for other manifestations of the disorder. Such a result may necessitate referral for proper counseling and medical guidance. In cases in which the personal or family history may suggest a specific genetic disease but no etiologic or likely etiologic variant is found, it is essential to communicate to the patient that CGES is not a comprehensive test for all disease-associated variants and that an absence of incidental findings does not mean that specific, indicated testing is unnecessary. Some laboratories routinely perform incidental-finding analysis, and others offer an opt-out for such an analysis; in either case, the ordering clinician should be aware of this issue and should obtain consent and counsel patients appropriately.

SUMMARY

CGES is a useful diagnostic test for a number of clinical situations, and it is already being used by clinical geneticists and other specialists. The indications and approaches we outline here are sure to evolve over time, as more data are generated for various clinical disorders, data interpretation is improved, and CGES is studied in new clinical situations (e.g., in neonatal medicine). Clinicians can feel confident ordering this test if they become comfortable with the evaluations, both before and after testing, and the processes that are required to maximize its usefulness, and if they are familiar with its limitations.

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REFERENCES

- Green RC, Rehm HL, Kohane IS. Clinical genome sequencing. In: Ginsburg GS, Willard HF, eds. *Genomic and personalized medicine*. London: Elsevier/Academic Press, 2013:102-22.
- Blue Cross and Blue Shield Association. Special report: exome sequencing for clinical diagnosis of patients with suspected genetic disorders. *Technol Eval Cent Assess Program Exec Summ* 2013; 28(3):1-4.
- Vassy JL, Lautenbach DM, McLaughlin HM, et al. The MedSeq Project: a randomized trial of integrating whole genome sequencing into clinical medicine. *Trials* 2014;15:85.
- ACMG Board of Directors. Points to consider in the clinical application of genomic sequencing. *Genet Med* 2012;14:759-61.
- 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.
- Mardis ER. The impact of next-generation sequencing technology on genetics. *Trends Genet* 2008;24:133-41.
- Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. *Cell* 2013;155:27-38.
- Altmüller J, Budde BS, Nürnberg P. Enrichment of target sequences for next generation sequencing applications in research and diagnostics. *Biol Chem* 2014; 395:231-7.
- Teer JK, Mullikin JC. Exome sequencing: the sweet spot before whole genomes. *Hum Mol Genet* 2010;19:R2:R145-51.
- Stein LD. An introduction to the informatics of "next-generation" sequencing. *Curr Protoc Bioinformatics* 2011;Chapter 11:Unit 11.1.
- Duzkale H, Shen J, McLaughlin H, et al. A systematic approach to assessing the clinical significance of genetic variants. *Clin Genet* 2013;84:453-63.
- Biesecker L. Improving the rigor of mutation reports: biologic parentage and de novo mutations. *Hum Mutat* 2012;33:1501-2.
- Rehm HL. Disease-targeted sequencing: a cornerstone in the clinic. *Nat Rev Genet* 2013;14:295-300.
- Johnston JJ, Biesecker LG. Databases of genomic variation and phenotypes: existing resources and future needs. *Hum Mol Genet* 2013;22:R27-R31.
- Bell CJ, Dinwiddie DL, Miller NA, et al. Carrier testing for severe childhood recessive diseases by next-generation sequencing. *Sci Transl Med* 2011;3:65ra4.
- MacArthur D, Manolio T, Dimmock D, et al. Guidelines for investigating causality of sequence variants in human disease. *Nature* 2014;508:469-76.
- Worthey EA, Mayer AN, Syverson GD, et al. Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. *Genet Med* 2011;13:255-62.
- Bainbridge MN, Wiszniewski W, Muddock DR, et al. Whole-genome sequencing for optimized patient management. *Sci Transl Med* 2011;3:87re3.
- ACMG Board of Directors. Points to consider for informed consent for genome/exome sequencing. *Genet Med* 2013;15:748-9.
- Wallace AJ, Watson CJ, Oward E, Evans DG, Elles RG. Mutation scanning of the NF2 gene: an improved service based on meta-PCR/sequencing, dosage analysis, and loss of heterozygosity analysis. *Genet Test* 2004;8:368-80.
- Hennekam RC, Biesecker LG. Next-generation sequencing demands next-generation phenotyping. *Hum Mutat* 2012; 33:884-6.
- Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med* 2013;15:565-74.
- Dorschner MO, Amendola LM, Turner EH, et al. Actionable, pathogenic incidental findings in 1,000 participants' exomes. *Am J Hum Genet* 2013;93:631-40.

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