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Defining the Clinical Value of a Genomic Diagnosis in the Era of Next-Generation Sequencing

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Abstract

As with all fields of medicine, the first step toward medical management of genetic disorders is obtaining an accurate diagnosis, which often requires testing at the molecular level. Unfortunately, given the large number of genetic conditions without a specific intervention, only rarely does a genetic diagnosis alter patient management—which raises the question, what is the added value of obtaining a molecular diagnosis? Given the fast-paced advancement of genomic technologies, this is an important question to address in the context of genome-scale testing. Here, we address the value of establishing a diagnosis using genome-scale testing and highlight the benefits and drawbacks of such testing. We also review and compare recent major studies implementing genome-scale sequencing methods to identify a molecular diagnosis in cohorts manifesting a broad range of Mendelian monogenic disorders. Finally, we discuss potential future applications of genomic sequencing, such as screening for rare conditions.

Keywords

clinical diagnostics; next-generation sequencing; whole-exome sequencing; whole-genome sequencing; genomic medicine; monogenic disorders

INTRODUCTION

Accurate diagnosis is central to the practice of medicine, enabling physicians to establish appropriate management strategies. For rare disorders that have a genetic etiology, accurate diagnosis takes on additional importance because of the potential hereditary nature of many such conditions. In clinical genetics, it is customary for patients and their family members to ask several fundamental questions about their condition: How did it happen? What can they expect? Is there a treatment or a cure? Who else in the family is at risk? Addressing these matters requires first answering the question, What is the diagnosis?

Although a clinical diagnosis can be achieved in some patients with genetic conditions, molecular testing allows the detection of disease-causing variants in order to establish a

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DISCLOSURE STATEMENT

J.S.B. is a principal investigator for the NCGENES study, which is investigating potential applications of genome-scale sequencing in a clinical setting.

specific genetic diagnosis. In some cases, follow-up testing in additional family members is needed to determine whether those variants were inherited or de novo (28, 80) and to identify other at-risk individuals. The application of genetic tests in patients who have a condition with a suspected genetic etiology can be fundamental to their care as well as that of other family members. The value of such information is often difficult to measure in terms of health outcomes (or narrowly defined as clinical utility) but frequently provides tangible benefits to the individual or family (sometimes described as personal utility) (1, 52, 60). Furthermore, establishing a specific genetic diagnosis allows patients and providers to transition from the so-called diagnostic odyssey, which can be expensive and burdensome, to a new focus on management (28, 57, 125). Thus, to some extent the clinical value of genome-scale testing is related to the diagnostic yield of the test or, conversely, to the ability of such testing to rule out a genetic etiology of disease.

The evolution of genetic sequencing technologies over the last decade has revolutionized the rate at which multiple genes can be simultaneously queried for sequence variants (132). This is predominantly due to the innovation of next-generation sequencing (NGS) technology that enables very high-throughput DNA sequencing (80). As a result, the trend in clinical genetics has been toward the use of multigene testing to achieve a molecular diagnosis in individuals with a genetic condition (132). These same technological advances have also facilitated the discovery of a large number of disease genes (7, 48, 116). We are now at a juncture where genomic technologies are advancing more quickly than we can resolve questions regarding which testing methods are most cost effective, efficient, and accurate. This is particularly true for genome-scale testing methodologies, which have only recently begun to be evaluated for the wide range of disorders encountered in the clinical setting (39, 68, 99, 120, 133, 134, 136).

This review summarizes the major recent studies that used genome-scale sequencing to establish a clinical molecular diagnosis in patients with presumed Mendelian conditions and evaluates the factors that influence diagnostic yield in such studies (39, 68, 99, 120, 134, 136). These studies highlight remaining challenges and questions regarding the use of genome-scale diagnostic testing in the clinic. Here, we address the following questions: (*a*) What is the clinical value of a molecular diagnosis, and is genome-scale sequencing a practical means to achieve a diagnosis? (*b*) What factors affect the diagnostic yield of genome-scale diagnostic testing? (*c*) What limitations exist for these approaches? (*d*) How will the clinical use of NGS affect the relationship between clinicians and molecular testing laboratorians? And (*e*) what does the future hold with regard to genome-scale sequencing?

CHOOSING THE RIGHT GENETIC TEST

When deciding the most appropriate testing method for a given patient, it is important to consider the strengths and weaknesses of each methodology (75). A clinician must develop a differential diagnosis and determine the most effective strategy to arrive at a specific diagnosis, ideally testing the most likely etiology first and reserving unlikely possibilities for later workup if necessary. In addition, the clinician may be inclined to prioritize testing for treatable conditions, even if the chance of the patient having those conditions is small. Given the many different tests used for diagnostic purposes in clinical genetics (reviewed in 60, 61,

64), clinicians must understand which conditions are adequately interrogated by any given test. For example, conditions caused by nucleotide repeat expansions are difficult to detect with the sequencing techniques discussed herein (7, 70, 122). In this review, we focus only on sequencing techniques and the scenarios in which they might be best implemented. Detailed reviews outlining the mechanics and biochemistry behind each sequencing technology, including those described below, can be found elsewhere (72, 80, 93, 100).

Single-Gene Tests

There are several situations in which multigene testing is unlikely to bring much added value over a single-gene test. Individuals suspected to have a condition with a single genetic etiology are ideal candidates for single-gene testing. A classic example of such a condition is cystic fibrosis, which is caused by pathogenic variants in only one gene, *cystic fibrosis transmembrane conductance regulator* (*CFTR*) (102). Targeted testing is also advantageous over multigene testing when a patient's clinical features are distinctive for a particular condition, such as Duchenne muscular dystrophy or neurofibromatosis type I. In these scenarios, single-gene testing is efficient and provides high clinical sensitivity and clinical specificity. Because single-gene testing typically employs Sanger sequencing (or first-generation sequencing), it is highly accurate but has traditionally been time consuming (80). A significant drawback to testing methods that rely on Sanger sequencing is its limited scalability, even when the technique is automated through the use of capillary electrophoresis and fluorescent dyes (80). The advent of massively parallel sequencing (also referred to as NGS) has drastically transformed the field of genetics, with current techniques generating terabytes of data (reviewed in 19, 70, 93, 122, 132).

Gene Panels

The ability to simultaneously query numerous genes by targeted NGS has led to the commercial availability of multigene diagnostic testing panels from clinical laboratories. This approach improves the analytical sensitivity of testing but may reveal findings in genes that are not directly relevant to the patient's condition. Conditions with significant genetic heterogeneity (such as neuropathy, in which more than 50 genes have been implicated) may benefit from the use of gene panels (104). Gene panel diagnostic testing in such a situation can decrease both the cost of testing and the time it takes to identify a diagnosis (or rule one out, if the test is truly negative) compared with traditional gene-by-gene testing (61).

As testing expands to include larger numbers of genes, there is an increased likelihood of identifying variants of uncertain clinical significance. Another potential disadvantage with this approach is the inherent variability of genome coverage. To ensure adequate coverage of the genes of interest, Sanger sequencing may be required to fill in regions of low coverage (95). Furthermore, determining which genes to include on such a diagnostic testing panel is not straightforward and requires setting a subjective threshold for the level of evidence necessary to consider a gene genuinely causative for the relevant disease or phenotype when altered. This issue is further complicated by the lack of standards available for evaluating a gene's association with disease coupled with the variability of evidence supporting disease causality from gene to gene. We discuss efforts to standardize this process further below (see Laboratory Thresholds for Returning Results).

Exome and Genome Sequencing

Sequencing the coding region of the genome, otherwise known as exome sequencing (7), has become a more prominent technique within the last several years, particularly as a gene discovery tool in the research setting (7, 44, 45, 48, 116). The Centers for Mendelian Genomics, funded by the National Institutes of Health to elucidate the genetic etiology of Mendelian phenotypes, have generated exome data for 16,226 individuals, discovered an association with disease for 647 genes using conservative disease causality criteria, and identified another 309 genes that are likely to be disease causing (28). A similar program in Canada boasts 67 newly identified disease associations from 250 cases (10), and European efforts have been equally successful (131).

As sequencing costs have fallen, exome sequencing has naturally been adopted more widely as a dia gnostic test. Several studies have now evaluated the use of exome sequencing in the clinical setting (39, 68, 99, 120, 133, 134). In many of these cases, exome sequencing is the last resort to obtain a molecular diagnosis; however, in certain scenarios exome sequencing may be more cost effective and beneficial to perform at the outset of diagnostic testing (110, 125). For conditions with significant genetic heterogeneity, first-tier exome sequencing could prevent the costly diagnostic odyssey. That being said, few studies have systematically compared the cost and efficacy of exome sequencing with those of traditional testing methods or evaluated the likelihood that exome sequencing would eliminate a diagnostic odyssey if implemented earlier in the diagnostic evaluation (9, 125). Despite minimal evidence to support the cost effectiveness of exome sequencing, in a survey of providers that ordered diagnostic exome sequencing for their patients, 74% of the respondents (34 out of 46) indicated that cost effectiveness was one of their reasons for ordering the test (5). More research in this area is warranted to determine whether genome-scale sequencing is an ideal early diagnostic step for all genetic conditions.

The American College of Medical Genetics and Genomics (ACMG) recommends implementing genome-scale sequencing—either exome or genome sequencing—only after more targeted testing is negative (96). As sequencing technology continues to improve, it is likely that more conditions will be amenable to testing via exome sequencing; however, there is evidence to suggest that in many cases a smaller gene panel will be sufficient and also more efficient than genome-scale sequencing (95). One group has recently suggested that there may be a middle ground between exome and gene panel testing: Mendeliome sequencing of all currently known disease genes, which would not allow identification of novel disease genes but would account for the vast majority of patient needs (109).

In many ways, the greatest challenge encountered when sequencing the exome is sifting through thousands of variants to identify one or two causative variants for a Mendelian disorder. Unlike single-gene or panel testing, in which results for variants of uncertain clinical significance are typically returned for any gene analyzed regardless of its consistency with the patient’s clinical presentation, such an approach to exome sequencing would result in unmanageable numbers of variants of uncertain clinical significance. The massive amount of variant data must therefore be filtered to obtain a smaller number of variants that provide a possible diagnostic etiology. This analysis requires that both the individual variant and the gene in which it resides must be properly interpreted, a process

that is significantly more efficient and thorough when molecular analysts are provided with detailed clinical information (76).

Genome sequencing provides many of the same benefits as exome sequencing, such as increased clinical sensitivity and potential cost effectiveness, but it also allows identification of deep intronic variants that could potentially alter splicing or repress transcription, as well as detection of structural genomic changes. One caveat of genome sequencing is that so little is understood regarding the intronic regions of the genome that the advantage gained is likely to be limited until noncoding genomic variants can be more effectively interpreted. The few studies that have compared exome and genome sequencing are insufficient to determine whether one technique is better than the other. For example, in six individuals with isolated congenital asplenia, genome sequencing was more effective than exome sequencing at identifying single-nucleotide variants (11), whereas in nine patients with intellectual disability, the techniques exhibited little difference in the variants identified (117).

WHAT IS THE CLINICAL VALUE OF A MOLECULAR DIAGNOSIS?

What is the value in identifying a molecular diagnosis for an individual's genetic disease? The answer depends on who is asking the question and how one defines value. A molecular diagnosis provides valuable information regarding disease mechanism, contributes to the known catalog of pathogenic variants, and can alleviate the burden on medical professionals by ending a diagnostic odyssey. In some cases, the result may suggest management recommendations, but even in cases where such management changes do not occur, one may ask how patients might benefit from such information. The ACMG recently published a policy statement advocating the position that identifying the genetic etiology of an individual's disease has utility for not only the patient, but also his or her family and society as a whole (1). This viewpoint is in contrast to the more narrow notion that for a genetic test to have clinical utility, it must directly affect the health outcomes of the individual being tested (55).

From the patient's perspective, a molecular diagnosis is of great interest and utility (13). Most patients and their families are extremely interested in knowing what disease they have, how it was caused, what the disease progression might be, whether treatment is available, and how the condition might affect other family members (13). Each of these questions can be addressed once a diagnosis is established, although the answer may not always be favorable. For example, identifying a pathogenic variant within a gene associated with Charcot-Marie-Tooth disease, a heterogeneous neuropathological disorder with variable phenotypic manifestations, may not enable specific treatment (20). Although only supportive management is available for this disorder, the specific type of Charcot-Marie-Tooth disease responsible for an individual's condition has a direct effect on the prognosis as well as the recurrence risk in the family, both of which vary depending on the molecular subtype (56).

Thus, the clinical value of genomic diagnosis depends on a chain of contingencies, including the value of information for the patient and his or her family, the diagnostic yield of the test performed, the effect of a positive or negative result on the management of the patient and

his or her family members, and whether this information ultimately leads to better clinical outcomes. To date, most studies have focused on the diagnostic yield of genome-scale sequencing, and these studies make up the core of this review.

FACTORS THAT INFLUENCE THE DIAGNOSTIC YIELD OF MULTIGENE TESTING

When establishing the diagnostic yield of a particular testing method, it is critical to establish the parameters of the test from both the clinical and analytical standpoints (Figure 1). The reported yield of testing is influenced by the technical performance of the test (analytical sensitivity and analytical specificity), the nature of the patient population being tested, the extent to which genetic etiologies are known for disorders represented in the patient population (clinical sensitivity), whether family members are concurrently being tested (to evaluate inheritance patterns), and the thresholds employed by the laboratory for variant interpretation and reporting. From the analytical perspective, it is critical to understand the methodology (including bioinformatic analysis) used within a laboratory to analyze the results, the technical limitations of a particular testing method, and how the laboratory director defines positive and uncertain results.

Patient Population: What Is the Likelihood of Identifying a Mendelian Condition?

It stands to reason that genetic testing in a highly selected population of patients with phenotypes indicative of Mendelian disorders will have a higher yield than testing in a population with possible phenocopies. For example, within a population of patients with suspected hereditary cancer susceptibility, there will likely be some whose cancer was sporadic and resulted from multifactorial etiologies rather than from a monogenic cancer predisposition syndrome. The same is true for conditions such as autism spectrum disorders (78). Furthermore, as understanding of human genetics improves, it is probable that mechanistic complexity will be discovered in more conditions, potentially resulting from variation within multiple genes as well as environmental factors (32, 115). Individuals with such conditions are less likely to benefit from genome-scale sequencing until scientific advances allow more accurate delineation of the extent to which genetic variants influence disease and more accurate interpretation of their combinatorial effects.

Another factor that influences yield is whether or not the patient population has had extensive prior diagnostic testing (110). Genome-scale sequencing of patients who are naive to genetic testing is expected at minimum to achieve a diagnostic yield consistent with that of traditional consecutive analysis of well-established disease genes. By contrast, if all known disease genes related to the patients' conditions have been effectively ruled out, then the yield of further genome-scale sequencing is likely to be low unless the study has been explicitly designed to discover novel candidate genes that may cause disease (22, 41).

The yield of genome-scale sequencing has been evaluated in cohorts of patients with specific disorders, ranging from cancer to intellectual disability to rare diseases such as Joubert syndrome (34, 40, 43, 114, 124). These initial studies clearly illustrate that some conditions are good candidate diseases for genome-scale testing; ophthalmological disorders, for

example, have a diagnostic yield over 50% (18, 69). By contrast, autism spectrum disorders may have a much lower yield; for example, a small study of 95 parent-proband trios had a yield of only ~8% (119). Interestingly, as investigators discover more disease genes and better understand the molecular etiologies of various inherited conditions, well-designed gene panels may ultimately become the ideal method for clinical analysis, given their high clinical sensitivity and limited potential for incidental or secondary findings. This diagnostic elegance would allow clinicians to focus on the diagnostic question at hand while avoiding the distraction of interpreting other genomic findings. Genome-scale sequencing could then be reserved for cases with negative panel testing but truly compelling evidence for a monogenic etiology for the disease.

Technical Limitations of the Tests

Limitations associated with the testing methodology directly affect the diagnostic yield. For example, false negative results can occur if a region relevant to the disease in question has poor or low coverage via the sequencing method used. This issue can be further complicated when using exome sequencing, which requires an initial enrichment step prior to sequencing that, depending on the exome capture efficiency, may not give full representation of some regions of interest. The NGS techniques that are currently most frequently utilized (which rely on short sequence reads) are also not optimal for detecting nucleotide repeat expansions (or other types of variants within repetitive sequence), large insertions or deletions, copy number variation, or intronic variants. This limitation is likely to be overcome by longer read lengths and/or enhanced bioinformatics tools.

Proper Informatics Infrastructure to Analyze Data

The massive amount of data generated by NGS necessitates computational assistance to produce a data file that is both meaningful and manageable. There are currently no established standards regarding data processing, an issue that is further compounded by the many different tools available for each of the data-processing steps that affect the variants that are ultimately manually reviewed (8, 70, 80). Extensive reviews of the bioinformatics tools used to analyze large sequence data sets can be found elsewhere (8, 89). Briefly, raw sequence reads are transformed into an annotated list of potentially interesting variants within disease-causing genes through a series of informatics processes: (*a*) quality assessment of the sequencing reads, (*b*) alignment or mapping to a reference genome, (*c*) variant calling, and (*d*) variant annotation (8, 80). Several bioinformatics tools are available for each of the steps in the computational process (89). Unfortunately, low concordance has been found for certain types of variant calls among five different pipelines (88, 89), suggesting the need for further optimization of NGS informatics processes to ensure the highest technical accuracy.

Laboratory Thresholds for Returning Results

Comparisons of different publications (whether from research groups or clinical laboratories) are confounded by inherent variability regarding the genes that are included on a panel test (or interpreted on exome or genome analysis) and the thresholds that govern which types of variants (or combinations of variants) are returned as possible diagnostic findings. Even with available guidelines for clinical NGS testing and variant interpretation,

there is subjectivity to the manner in which different groups utilize and implement these guidelines.

For targeted NGS gene panels, the ACMG recommends including only genes with a role in disease that has been sufficiently supported by scientific evidence, excluding genes without a validated role in disease (96). Prior to 2016, a systematic method for evaluating the strength of evidence supporting a gene's relationship with disease did not exist, and thus each laboratory may have interpreted "sufficient evidence" differently. As such, laboratories including more genes in their analysis may claim higher diagnostic yields despite the inadequate evidence supporting a role in disease for some genes. The National Institutes of Health–funded Clinical Genome Resource (ClinGen) consortium aims to develop an open-access resource for clinically relevant genes and variants and is establishing a framework to define and evaluate the clinical validity of gene-disease pairs (97). It is anticipated that these standards will aid in the creation of professional recommendations regarding which genes should be considered for diagnostic testing, leading to more uniform thresholds for the return of results.

A second layer of complexity and subjectivity comes with interpretation at the variant level, which can differ between accredited laboratories for the same variant (90). The recently updated ACMG guidelines for assessing variant pathogenicity are more structured and systematic than previous guidelines, which may lead to greater consistency in the use of different types of evidence (101). There remains a certain degree of nuance in several of the rules, which may require specification for individual disease areas or individual genes. The threshold that differentiates categories of pathogenicity is also subjective, leading to potential discrepancies between groups regarding variants that are considered to fall into the category of uncertain significance as opposed to likely pathogenic or likely benign (Figure 2a). How a laboratory defines their threshold for pathogenicity (conservative or lenient) influences whether they report a finding as a positive or as a possible/uncertain result, which in turn directly influences the yield of results that are returned.

Analysis at the case level integrates the variant data with the patient's clinical information to determine whether the diagnostic test has identified a definitive, likely, or possible explanation. As with variant interpretation, labs may have differing interpretations of an overall case based on the degree to which the phenotype matches that of the patient or how judicious a laboratory is regarding phenotypic expansion. Another critical factor is whether the laboratory requires phase or de novo status to be known in order to match the inheritance pattern of the condition indicated by the variant data (Figure 2b). The laboratory's interpretation is further complicated by the post-test clinical correlation that naturally occurs when the provider compares the variant data with the patient's clinical picture. In some cases, further workup by the physician may show that a result initially reported as positive is actually irrelevant to the patient's condition. Thus, integrated research studies that follow patients through enrollment, analysis, return of results, and clinical follow-up may provide a diagnostic yield that results from a different level of analysis than those reported by diagnostic laboratories.

WHAT IS THE CURRENT DIAGNOSTIC YIELD FOR EXOME AND GENOME SEQUENCING?

In 2009, second-generation sequencing technology was successfully implemented to query the exomes of a small number of individuals with a specific condition for disease-causing variants (27, 85). Since then, reports of exome and genome sequencing analysis for diagnosis (84) and/or gene-disease relationships (7, 10, 44, 45, 66, 94, 116) in particular disease cohorts have proliferated in the literature (30, 34, 36, 49, 69, 108, 118, 121, 124, 135). Research groups and clinical laboratories have rapidly implemented exome sequencing, and within the past few years, results from several cohorts have begun to demonstrate the full potential of this technique as a diagnostic tool in the clinic across a broad range of phenotypes (39, 68, 99, 120, 133, 134, 136). In the following sections, we review, compare, and contrast these recent studies to establish what makes an exome study successful.

Summary of Major Recent Studies

Tables 1 and 2 summarize the demographic information and diagnostic yields of selected genome-scale sequencing studies in which a broad range of patients were analyzed through either research projects (120, 136) or Clinical Laboratory Improvement Amendments (CLIA)–approved molecular diagnostic laboratories (39, 68, 99, 134); for comparison we have also included a preliminary analysis of 575 (of more than 650) patient exomes that our research group is currently preparing for publication, (J.S. Berg, N.T. Strande, G. Haskell, B. Powell, A.K.M. Foreman, et al. manuscript in preparation). This summary cannot possibly account for all case studies or small cohorts that have been published, and instead focuses on larger cohorts (>100 probands) in which exome sequencing or genome sequencing has been performed in a primarily diagnostic context, in order to draw broad comparisons and potentially generalizable conclusions about the diagnostic yield of genome-scale sequencing.

Zhu et al. (136) reported exome sequencing results from 119 individuals, including reanalysis of 6 negative cases from an earlier pilot study (82). Patients included in this study were chosen to represent the heterogeneity of clinical features present in a genetics clinic. Patients were recruited from either the Genome Sequencing Clinic at Duke University Medical Center or the pediatric clinic at the Sheba Medical Center in Israel. After a genetic evaluation and traditional diagnostic testing did not provide a molecular diagnosis for each participant, trio sequencing was performed. For each case, genotypes that were considered to qualify as a potential answer were required to meet several criteria dependent on multiple factors, including inheritance pattern and minor allele frequency (for additional details, see 82). Because the analysis was not first filtered by gene, each potential genotype was subsequently reviewed to determine whether the gene in question is associated with a disorder consistent with the phenotypic presentation and inheritance pattern observed in that participant. Prior to classifying a case as resolved, both the genetics team and treating clinicians were required to agree on the final diagnosis, and the qualifying variants were then confirmed via Sanger sequencing in a CLIA-approved laboratory.

The North Carolina Clinical Genomic Evaluation by Next-Generation Exome Sequencing study at the University of North Carolina at Chapel Hill (UNC) is part of the National Human Genome Research Institute–funded Clinical Sequencing Exploratory Research consortium (http://cser-consortium.org). This study has enrolled more than 650 patients with a broad spectrum of health conditions in which the referring physicians (geneticists, neurologists, cardiologists, and oncologists) thought that exome sequencing might be useful in determining the etiology. Analysis was performed as proband-only exome sequencing with Sanger sequencing confirmation of all positive results and family studies in selected situations (when available) to determine phase, segregation, or de novo status. An important aspect of this study was to ensure participant diversity (including age); therefore, the researchers decided that trio testing was unlikely to be practical in this study (i.e., because parental samples are likely unavailable for older participants). We include here a preliminary analysis of 575 exomes completed at the time of this review; the exomes of the complete cohort are currently in preparation for publication (J.S. Berg, N.T. Strande, G. Haskell, B. Powell, A.K.M. Foreman, et al. manuscript in preparation). Variants qualifying for return of results met a relatively high threshold contingent on criteria consistent with the ACMG sequence variant interpretation guidelines, and the research group identified several distinct categories of uncertain case-level results.

Lee et al. (68) reported exome results for 814 participants who were not required to have any prior diagnostic testing conducted. Geneticists referred the majority of patients, and thus many cases did have extensive prior genetic testing. Referring providers were able to choose between proband testing only or trio exome sequencing. Exome variants were filtered using patient-specific gene lists generated using key phenotypic terms provided by the physician. A bioinformatician presented qualifying variants to a review board comprising a diverse set of professionals, including physicians, pathologists, geneticists, genetic counselors, and bioinformaticians. Confirmation via Sanger sequencing was performed in the first 300 cases and thereafter only when quality metrics were not met.

Yang et al. (134) recently reported results from exome analysis of 2,000 participants. This study was a continuation from their pilot study published a year earlier, which reported the results of an exome analysis of 250 individuals (133). Selection criteria were not stipulated by the clinical laboratory, which allowed referring physicians to use their own discretion to determine whether a given participant was an appropriate candidate for exome sequencing. Samples were obtained from the proband and both parents, but exome sequencing was conducted only on the proband. Parental samples were subsequently used to determine variant phase, inheritance pattern, and conduct segregation analysis of individual candidate variants. Qualifying genotypes were required to meet a set of minimum criteria prior to discussion with the laboratory directors and appropriate clinical experts.

Farwell et al. (39) presented exome results for 500 patients referred by physicians. All referring providers were asked to supply the laboratory with pedigrees, proband clinical information, and blood samples for the proband and all available relatives. For each case, a molecular geneticist or genetic counselor reviewed the clinical and family history. After annotation, two molecular analysts, a molecular geneticist, and a genetic counselor analyzed

potentially causative variants; the potentially causative variants were confirmed via Sanger sequencing.

Retterer et al. (99) reported results for 3,040 individuals referred for exome sequencing. Selection criteria for clinical exome sequencing were determined by referring clinicians, who were asked to provide the laboratory with phenotypic information and blood samples from the parents (if available) in addition to those from the proband. Qualifying variants were confirmed, and follow-up testing was conducted on additional samples if provided. From the described methods, it is not clear whether variants were discussed with a group of professionals, as in other studies.

Finally, Taylor et al. (120) reported results for whole-genome sequencing of 156 probands. Participants in this study were selected if there was a strong suspicion of genetic etiology based on family history and if previous diagnostic testing had not been fruitful (for details, see 120). Trio sequencing was conducted in a small proportion of the participants for which samples were available. All proband samples were available to the laboratorians for additional testing and analysis if necessary. Once potential causative variants were identified by analysts, results were confirmed via Sanger sequencing and the information was returned to the clinicians, who were responsible for deciding what to do with the information.

Comparison of Overall Diagnostic Yield Across Studies

Table 2 summarizes the diagnostic yield of positive, uncertain, and negative results across the seven studies described above. The mean diagnostic yield of positive results across all of the studies was 26.4% (range 16.9–30.4%). When trio sequencing was performed in the clinical studies, the diagnostic yield increased to over 30% (31.0–37.3%). The yield reported for presumed deleterious variants in candidate genes in the available clinical studies was consistently low (3.8–7.6%). Although the yield of possible/uncertain results was not always clearly delineated, the mean yield of such results among the studies in which this category could be determined was 39.8%; interestingly, there was a very wide range in the percentage of possible/uncertain results (8.6–51.8%). We suspect that this variability reflects different laboratory thresholds for calling variants likely pathogenic or likely benign, or different ways of classifying case-level results.

Although there is a good representation of phenotypic variety among the four clinical exome studies, intellectual disability was a clinical feature in 42.8% of the cases across these studies. The highest yield of all phenotypic categories (comprising more than 15 individuals) consistently observed across the studies was in ophthalmological cases [32.4–48%; ophthalmology was not specifically reported by Yang et al. (134)]. The most consistently reported phenotype with the lowest diagnostic yield was cancer [4–19%; cancer was not specifically reported by Yang et al. (134)].

Factors Influencing Diagnostic Yield Between Studies

As previously described, there are many different criteria that can ultimately impact the overall diagnostic yield of a genomic study. Here we summarize the criteria likely contributing to the differences in diagnostic yield observed amongst the seven genome-scale studies reviewed above.

Proband versus trio testing.—Data from family members (e.g., parental studies or trio testing) are important for determining phase, because short reads do not often allow for unambiguous determination of whether two variants identified by NGS are in a *cis* or *trans* configuration or whether the variant occurred de novo. The studies described here used different strategies for parental testing. All of the cases in the Zhu, et al. study (136) included the exomes of the proband and both parents (i.e., trio-exome sequencing), which were evaluated together to aid in identifying de novo variants. In contrast, the UNC study used a proband-only design owing to funding limitations and the imperative to acquire a larger study population for subsequent qualitative and quantitative psychosocial research. Parental studies were obtained as needed (when available) to adjudicate phase or de novo status. In the reports from clinical laboratories, diagnostic yield did seem to be influenced by the ability to interrogate parental variant data, with a higher yield in trios compared with proband-only tests (39, 68, 99, 136) (Table 2). Although sequencing a trio increases the overall cost of the test, the yield in each of these studies was higher by 8–17% than proband-only testing (Table 2), suggesting that trio exomes should be performed when possible to obtain the highest possible yield.

Age of participants.—The average participant age reported in these studies ranged widely between groups (7.7–32.8 years), with all but one study reporting an average participant age below 18 years (Table 1). Not all of the studies included specific details regarding the population studied; for example, two studies did not include a breakdown of their study population by age or gender, and two additional studies only provided ages for those individuals that received a molecular result and thus the age statistics are based on that information (Table 1). Based on the demographic information in the reports, ~75% of the reported participants were children, which suggests that the predominant population that is currently being referred for exome sequencing is pediatric. By contrast, ~66% of the participants in the UNC research study were adults.

When evaluating the adult population alone, the overall diagnostic yield averaged ~21% (range 16–24%) across all studies except the Retterer et al. study (99), which did not report a yield specifically in adult patients. Interestingly, Posey et al. (91) recently published results for an adult-only cohort in which the diagnostic yield was 17.5% (85 out of 486), which is consistent with the lower yield observed at UNC and suggests that the conditions represented among adults undergoing genetic testing may be less amenable to genome-scale diagnostic testing. The reasons for this difference are not clear, but they could be related to the extent of prior genetic testing of these patients (e.g., hereditary cancer or neuropathy), which would have depleted patients with pathogenic variants in known disease genes from the cohorts being studied. Alternatively, there may simply be more phenocopies of the conditions being examined in the adult population.

Classification of overall case results.—The studies reviewed here each utilized rubrics to define a positive result that took into account the pathogenicity of the variant(s), the degree of consistency between the phenotypic features of the patient and the disorder indicated by the genetic findings, and whether the zygosity of the variant(s) fit with the inheritance pattern of the disorder. Owing to the limited descriptions of these rubrics, we

were unable to assess precisely how closely the criteria overlapped. Thus, it is not possible to directly compare the yield of positive results reported in these studies relative to the yields of possible/uncertain diagnostic results. Figure 2b shows the rubric used in the UNC study.

Some studies included additional, more stringent criteria in their determination of a positive case. For example, in the report from Zhu, et al. (136), the treating clinician had to agree that a particular variant could potentially explain the patient's phenotype in order for the case to be considered resolved. Similarly, the UNC study integrated post-test data from clinical follow-up studies that resolved some results that were initially possible/uncertain to either positive or negative. Thus, at least some of the difference in diagnostic yield between studies could be due to the opportunity to integrate clinical correlation after the laboratory result.

Another factor affecting the reported diagnostic yield is whether variants in candidate genes were considered as providing a positive or possible/uncertain case-level result. For example, the Taylor, et al. (120) considered compelling variants in candidate genes to be positive results when additional supporting data were available. In the Zhu, et al. study (136), a de novo variant in a candidate gene that could explain the patient's phenotype was defined as a possible/uncertain result that could potentially provide a genetic explanation. The Lee, et al. (68), Farwell, et al. (39), and Retterer, et al. (99) studies included novel candidate gene results in a category separate from their positive and possible/uncertain results. The UNC study did not evaluate or return any candidate genes as part of the diagnostic evaluation, but instead reserved those results for subsequent analyses that would only be returned as research results. Yang et al. (134) used a similar process in which a provider can request an expanded report that includes variants of uncertain significance in disease genes apparently unrelated to the patient's phenotype, or novel deleterious variants (e.g., truncating) in other genes not yet associated with disease.

Comparison of Diagnostic Yield Across Phenotypes

In addition to differences in the case-level interpretation across studies, there is considerable variation in the manner in which diagnostic results are categorized across phenotypic categories. In some cases, conditions can be intuitively grouped into similar disease categories (hereditary cancer, retinal disorders, nonsyndromic intellectual disability, etc.), but many childhood genetic syndromes include variable neurological phenotypes (hypotonia, seizures, developmental delay, etc.) along with dysmorphic features and/or congenital birth defects. Different groups may count the same disorder in different disease categories, making comparisons between reports challenging. Almost all of the studies reviewed here included an intellectual disability cohort (which may or may not have included seizure disorders) as well as a neurological cohort. Yang et al. (134) utilized a single category for multisystem disorders, whereas Retterer, et al. (99) broke down its yields into relatively specific diagnostic categories. Table 3 shows the descriptive terms utilized by each group. Ultimately, it will be valuable to have detailed case-level phenotypic information on patients reported in such studies in order to facilitate cross-study comparisons using standardized resources such as the Human Phenotype Ontology (63).

Conclusions About the Diagnostic Yield of Clinical Exome Sequencing

Together, these studies illustrate several important points regarding the implementation of genome-scale testing in the clinic. Comparing results across different studies requires a great deal of detail from each publication, and this detail is not always available. The overall diagnostic yield of exome studies appears to be slightly greater than 25% for individuals who have undergone previous diagnostic testing. These studies do not reflect what the yield would be in a naive population that has not previously undergone diagnostic testing, or how this yield would compare with available gene panels. It is clear that not all conditions and participants are ideal candidates for exome sequencing, but further research is needed to determine whether large diagnostic panels or genome-scale sequencing will be most effective for different disease categories.

CHALLENGES THAT ARE MORE PROMINENT IN GENOME-SCALE DIAGNOSTICS

What Are the Reproducibility and Comparability of Exome and Genome Results?

The implementation of genome-scale diagnostic testing in the clinic brings with it a set of challenges that are predominantly associated with this testing methodology. For example, direct comparison among exome and genome studies is limited for the reasons discussed above. The tendency to provide incomplete details regarding the computational analysis of large data sets makes comparison particularly difficult and is likely exacerbated by constraints set forth by publishers (83). Table 4 illustrates some of the missing or limited details regarding study design for the studies presented in this review. The lack of clinical details for patients reported in these studies and the inability to access the raw variant data because of privacy concerns further limit the ability to compare results across studies with similar clinical phenotypes. Exclusion of critical details coupled with inherently unique populations reported in each publication virtually guarantees a lack of reproducibility among these studies.

As NGS technology becomes more prominently utilized for diagnostic genetic testing, it will be important for each NGS study to systematically report the computational details, variant data, and phenotypic details necessary to replicate the study results. This information has been difficult to track in the past; fortunately, there are now integrative frameworks that allow the parameters and settings used when transforming the data to be automatically tracked and even imported directly into a text-editing program when preparing a manuscript (47, 53, 65, 71, 84, 98). Furthermore, many different groups have put forth best practice guidelines for properly implementing this technology, including the computational analysis of the data (38, 42, 59, 73, 107, 128). Such guidelines will ensure that key components of exome and genome analysis are conducted in a well-documented manner that will facilitate reinterpretation of results or potentially combination with other studies for meta-analysis, and will greatly enhance the utility of such information. Naturally, the privacy of the patient information will be an important concern as more data sets contain richer genotypic and phenotypic data. Case-level databases are being developed (67, 97) that may enable researchers and clinical laboratories to share more complete data sets in order to ensure

more complete comparisons while taking into consideration proper safeguarding of patient information.

Incidental and Secondary Findings

Genome-scale sequencing inevitably brings the potential to discover unanticipated findings. This matter was addressed in the December 2013 report of the Presidential Commission for the Study of Bioethical Issues (127), which defined incidental findings as results that are happened upon while searching for a diagnostic result, and secondary findings as additional results that are intentionally sought (see table 1.2 in Reference 127). The identification of such findings poses an ethical and practical dilemma when evaluating an individual's exome or genome. Over the last decade, this topic has been the center of significant investigation and discussion, particularly with regard to which results should be returned (21, 38, 46, 50, 62, 74, 77, 112, 130). As such, the ACMG has put forth guidelines outlining the types of results that should be reported to patients during clinical genome-scale diagnostic testing (51). There is still debate over which specific results should be returned, whether they should be intentionally sought, and how decisions should be made regarding various categories of secondary or additional findings.

Several groups have begun to address the types of results (in terms of variants, genes, and diseases) that should be returned to patients (15, 16, 46, 74). One approach is to consider the concept of actionability as a way to prioritize the analysis of potential incidental and secondary findings. Given that there are different degrees of actionability, some subjectivity will be inevitable when deciding the thresholds for return of results. Berg et al. (16, 17) proposed a semiquantitative metric to define the actionability of gene/disease pairs as a way to calibrate the potential benefits versus the potential harms associated with returning that information to the patient. This approach can be supported by an informatics structure that facilitates prioritization of variants based on these bins and provides a manageable workload for the molecular analyst (14).

SHIFTING ROLES BETWEEN CLINICIANS AND LABORATORIANS

As genome-scale sequencing becomes accepted as a diagnostic tool in the clinic, a certain degree of overlap is expected between the role of the clinician and the role of molecular diagnostic laboratory professional in evaluating molecular results. Focused gene panels targeting a specific diagnostic spectrum allow a diagnostic laboratory to reasonably assume that the ordering provider has identified certain phenotypic characteristics in a patient that suggest a condition within that spectrum. Thus, the variants identified in the assay can be reviewed and reported based on the assumption that they may have relevance to that individual's phenotype. As panels become more expansive and encompass a broader range of phenotypes, this assumption may no longer be correct, which could result in reporting of variants (especially variants of uncertain significance) that are essentially irrelevant to the patient's condition. This would require additional interpretation and counseling on the part of the ordering clinician, with the potential for misunderstanding (by clinician or patient) and errors in clinical management. At the scale of exome or genome sequencing, the conceivable number of reportable variants becomes enormous, requiring laboratories to set

thresholds for returning results based on the likelihood of the variant's pathogenicity and the degree to which that variant is consistent with the patient's phenotype and expected inheritance pattern. In such situations, detailed phenotypic information is crucial for filtering the list of potential candidate variants to ultimately differentiate those likely responsible for the phenotype from those that represent unrelated secondary findings.

Traditionally, many genetic testing laboratories have requested details regarding patients' phenotypes and family histories using forms completed by the clinician. There is virtually no standardization of the information content or the qualifications of the individuals completing the forms. Furthermore, after variant results are returned and subjected to clinical correlation by clinicians who are involved in the patient's care, opportunities for communicating additional information to the laboratory for iterative interpretation of the variant data are limited. In fact, comparison of the overall interpretation of 93 clinical exome sequencing results between laboratories and clinicians demonstrated that when discrepancies occurred (which they did in approximately 10% of cases), they often involved additional clinical information that was subsequently considered in the interpretation of the result (113). As genome-scale sequencing becomes more common in medical care, it is imperative that laboratories and clinicians develop standards for communication about phenotypes and genotypes (both the presence and absence of specific findings) and that formal channels are made available for back-and-forth discussions. As the boundary between molecular analysis and clinical interpretation becomes increasingly blurred, it will be important to establish what is expected from each profession and how that may change depending on the clinical scenario.

In other areas of medicine, such as radiology and pathology, a similar overlap in roles has evolved. A recent study in Belgium found that 96.8% of the general practitioners surveyed (273 out of 282) regard the radiology report as a valuable resource to guide their interpretation of the radiograph results (23). These practitioners considered the report to be "an indispensible tool" and also thought that the radiologist's clinical knowledge of the patient's condition and the medical context for the imaging is imperative to produce a quality report (23). Similarly, in pathology there is a need to effectively communicate complex information in order to avoid misinterpretation (92) and maximize comprehension (105).

Given the potential benefit of a healthy and active relationship between laboratory professionals and clinicians, the Association for Molecular Pathology encourages and recommends such interactions (111). The dynamic between laboratory professionals and clinicians will continue to evolve as sequencing technologies advance into clinical care. At one extreme, laboratories may require extensive information up front, including access to clinic notes, imaging, and other medical records, in order to assemble a phenotypic pattern that they can use to prioritize and analyze variants and to determine which to report back to the clinician. At the other extreme, laboratories could provide comprehensive variant data, extensively annotated and interpreted without prior assumptions about diagnostic relevance, which would then be processed in the context of the patient's detailed information by a clinician with expertise in genomic medicine. We would argue that the optimal arrangement for patient care is somewhere in between, with collaboration between laboratories and

clinicians leading to the most accurate interpretation of genomic sequencing results. Of course, there are numerous barriers to this theoretical “optimal” practice, including questions about the infrastructure required to facilitate such extensive collaboration, reimbursement for the time and cognitive services required for this collaboration to occur, and the training that would be required for both parties to effectively contribute.

WHAT DOES THE FUTURE HOLD FOR EXOME AND GENOME TESTING?

The dramatic reduction in the cost of sequencing over the past decade has naturally led to calls for genome-scale sequencing to be integrated into the routine of health care as a resource to be consulted throughout an individual’s life. This extension of clinical genomic medicine holds great promise for identifying individuals at increased risk for health problems but requires careful investigation before the benefits would outweigh the harms (126), including better data on the frequency of potentially reportable findings (2) and the downstream impact of this information on health care utilization and outcomes (12, 33, 54). The potential expansion of genomic screening in newborns and children raises additional ethical and social issues (3, 24, 29, 103), including the child’s “right to an open future” (25, 33, 35, 79). Clearly, widespread implementation of genome-scale sequencing in children would require an infrastructure to enable informed decision making by parents regarding the categories of information that they choose to learn, perhaps in the form of evidence-based sets of genes to be examined at particular developmental time points and integrated into routine well-child care.

In any age group, the use of genomic sequence data in a predictive context leads to inherent challenges regarding its predictive value. Clinical interpretation of common variants associated with multifactorial disease is confounded by the very small contribution made by any single variant and the difficulties inherent in providing robust predictions at an individual level (58). Interpretation of rare variants is equally challenging, because the prior probability of a rare Mendelian disorder is very low, and variants with less than 99% certainty of being pathogenic will inevitably generate false positive results. Moreover, ascertainment bias inherent in rare disorder studies skews the apparent penetrance and expressivity of these conditions by selecting for the most extreme phenotypes or the highest-risk families. As a result, even truly pathogenic variants may lead to overdiagnosis and subsequently unnecessary downstream medical interventions. These challenges are not impossible to overcome, but doing so will require much more research in order to justify widespread adoption of genomic screening across the entire population.

Sequencing technology is also being rapidly adopted in the prenatal setting, where noninvasive screening can now be performed on fetal DNA isolated from the mother’s blood sample (86). Currently, this technique is limited to detecting aneuploidy and large copy number variants, but it may only be a matter of time until the techniques used to isolate and sequence fetal DNA can be used to generate high-quality single-nucleotide variant calls and thus to screen for virtually all types of genomic alterations. Prediction of disease in the prenatal context will be fraught with the same problems of false positive results and overdiagnosis. The inevitable reproductive decision making that must take place in the face of abnormal results would make this an even more high-stakes endeavor, requiring

significant genetic counseling services and putting substantial pressure on parents to make decisions and act on genomic information (if desired) in a relatively short period of time. These factors make it unlikely that genome-scale analysis will be broadly implemented in routine prenatal care in the near future, although some boutique providers may offer such services to patients who can pay out of pocket and wish to have the largest amount of information possible during pregnancy.

At the other end of the life spectrum, there is also interest in using genomic technologies to establish specific molecular etiologies for unexplained death, as in the case of sudden cardiac death (81, 87). This information can be of much more than academic value; families of the deceased may learn actionable information that would allow other family members to prevent a similar outcome or reduce the possibility of an inherited risk. In the research setting, consent documents often do not address what should happen with genomic information if a patient or research participant dies. Early research in this area suggests that individuals feel some obligation to share genomic results with their relatives and would want their relatives to have access to this information after their death (4, 26). However, unique challenges and ethical concerns might arise regarding which family members could be authorized to request postmortem analysis and learn the results (especially when the legal next of kin may not have a personal stake or interest in the information) (106, 129).

As with any new technology in health care, genomic sequencing has the potential to revolutionize medicine through more individualized risk prediction and management options (6, 37). In some respects, personalized medicine is already at hand, with improved genetic testing now able to establish specific molecular diagnoses in a substantial proportion of patients with suspected Mendelian disorders. The move from diagnostic testing in an affected population to broad use of genomic analysis in the general population must be accompanied by further research on the needs of patients and providers for education and informed decision making, preference setting for different categories of genomic information, evidence-based analysis of genomic variants, and better integration of genetic results into the electronic health record and patient portals (123).

Genome-scale sequencing that provides a diagnostic answer will transform the care of substantial numbers of patients suspected to have rare Mendelian disorders, some of whom will be able to take advantage of management options that improve their outcomes. Even if this information does not result in tangible changes in disease course, families will be able to transition from the diagnostic odyssey to focus on understanding the condition and proceeding with long-term management. The results of the studies reviewed here should provide a strong foundation for further investigation regarding the utility of genome-scale sequencing in clinical diagnosis.

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DEFINITIONS

1. **Analytical sensitivity** – A measure of an assay's ability to detect genetic variants that are present in an individual (technical true positive results).
2. **Analytical specificity** – A measure of an assay's tendency to identify genetic variants that are not actually present in the individual (technical false positive results)
3. **Candidate gene** – A gene that is not currently associated with human disease, but that experimental evidence indicates could be disease causing
4. **Clinical sensitivity** – A measure of the test's ability to identify the correct diagnosis in a particular patient population (clinical true positive results).
5. **Clinical specificity** – A measure of the test's tendency to identify an incorrect diagnosis in a population (clinical false positive results).
6. **Diagnostic yield** – The rate at which a particular diagnostic test establishes a molecular diagnosis in a population of patients.

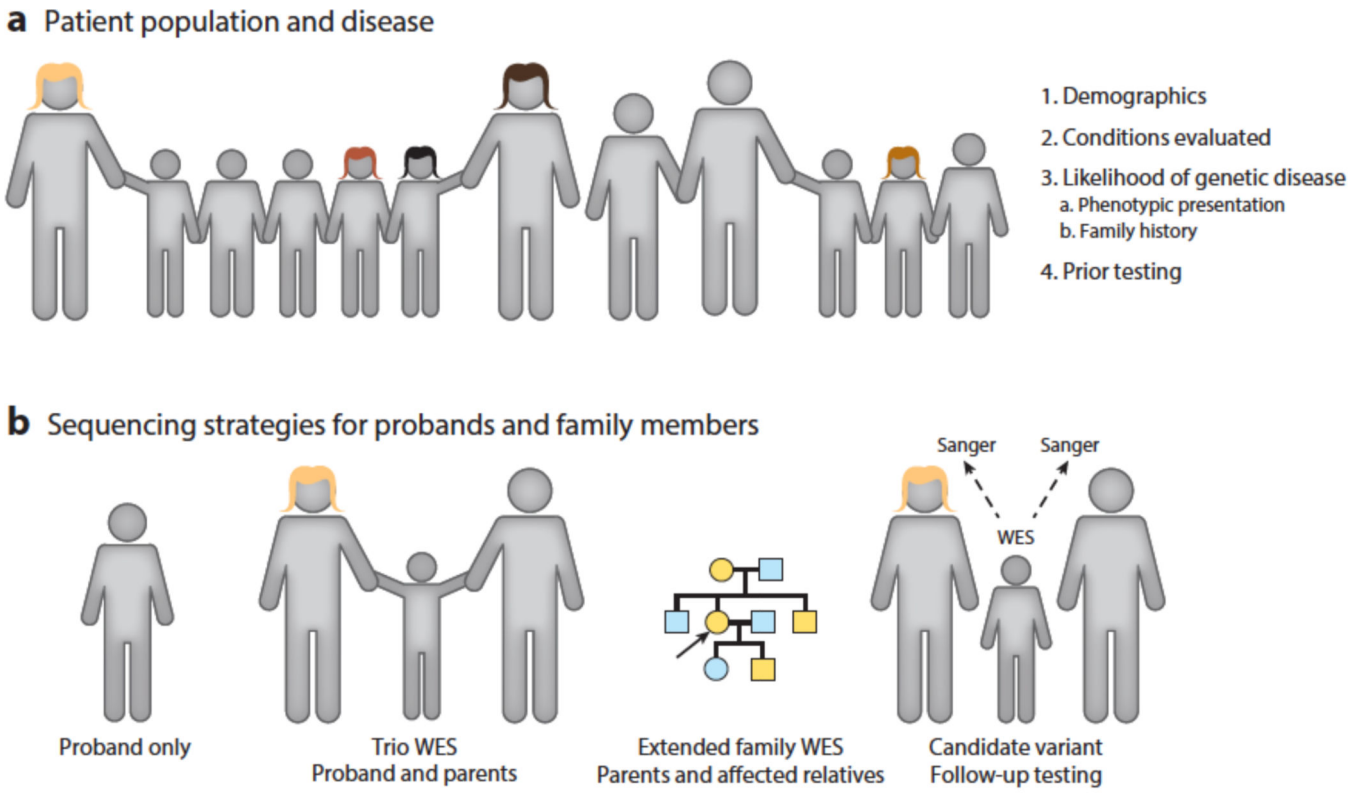

Figure 1:

Factors that influence the diagnostic yield of multigene testing. (*a*) Characteristics of the cohort being tested that influence the yield of diagnostic testing include demographic information, the conditions included in the cohort, the likelihood that the individuals being tested have a genetic disease, and any prior diagnostic testing. (*b*) The diagnostic yield varies depending on the amount of information provided by the sequencing strategy, which may be proband only, trio whole-exome sequencing (WES), extended family WES, or candidate variant follow-up with Sanger sequencing. (*c*) Aspects of the sequencing approach and data analysis that influence yield include the DNA capture method for exome sequencing, sequencing platform, alignment software, and prioritization of variants (gene lists derived from a general phenotype as opposed to patient-specific phenotypic information).

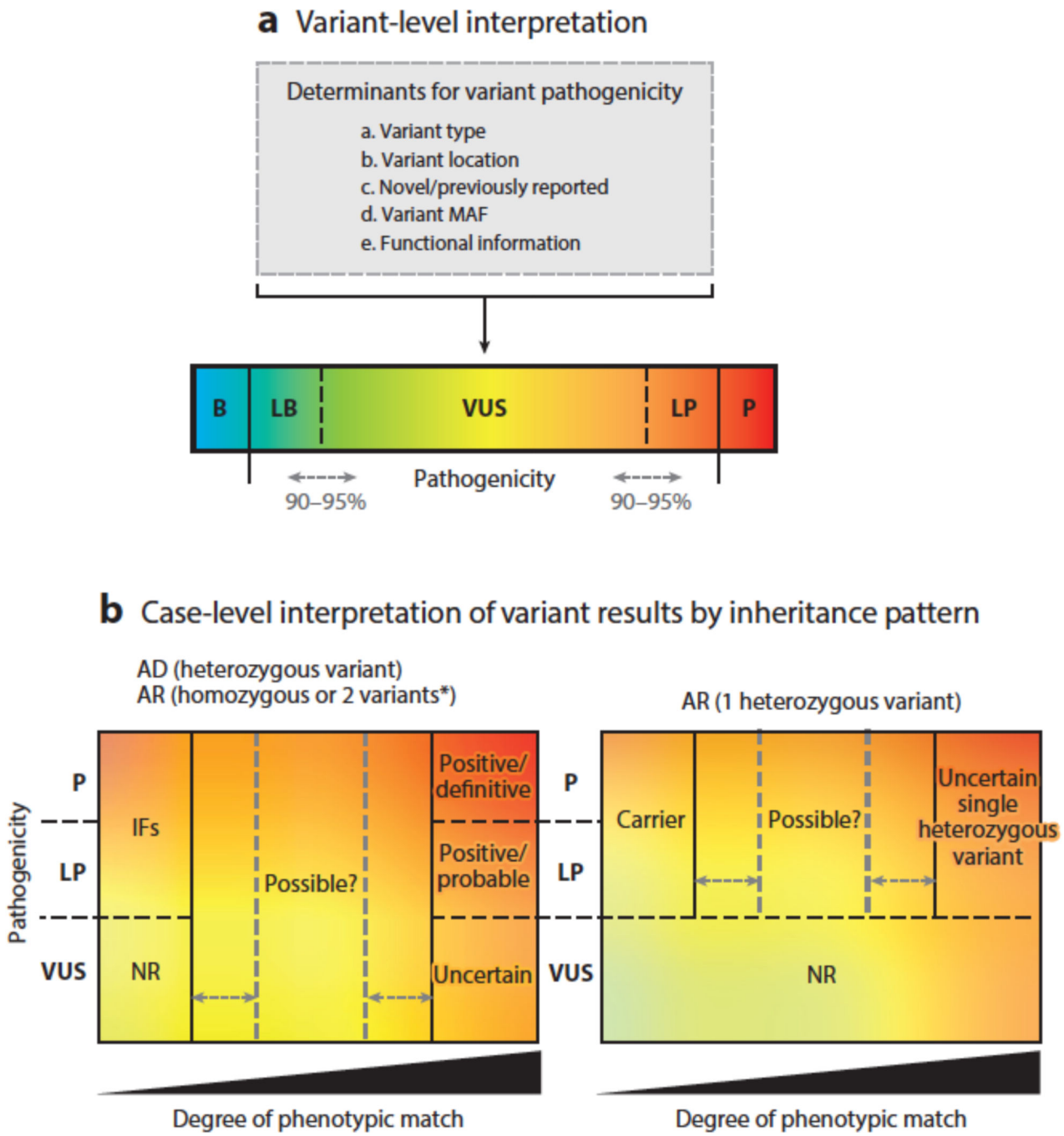

Figure 2:

Variant-and case-level interpretation. (*a*) Variant-level interpretation is influenced by multiple criteria that determine the pathogenicity of the variant. The color bar depicts the subjectivity involved in assigning a level of pathogenicity to variants, particularly with regard to the boundaries of the variant of uncertain significance category. The dashed lines and arrows represent the different thresholds that may be used by various groups to assign pathogenicity assertions. (*b*) Case-level interpretation incorporates both the pathogenicity of the variant(s) and how well the variant or combination of variants matches the patient's

phenotype. Whether results are returned to a patient depends on the inheritance pattern of the condition of interest. The dashed lines and arrows indicate subjectivity regarding the boundaries of these categories among different groups. The asterisk indicates that next-generation sequencing may not be able to identify whether two variants identified in a given gene are in *cis* or in *trans*. When the phase is unknown, the case-level result may be reported as an uncertain result until compound heterozygosity can be confirmed.

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; B, benign; IF, incidental finding; LB, likely benign; LP, likely pathogenic; MAF, minor allele frequency; NR, not reported; P, pathogenic; VUS, variant of uncertain significance.

Table 1

Demographic information for seven large genome-scale studies

Study type		**Exome (research study)**		**Exome (clinical laboratory)**				**Genome**
Study		**Zhu et al. (136)**	**NCGENES**[a]	**Lee et al. (68)**	**Yang et al. (134)**	**Farwell et al. (39)**	**Retterer et al. (99)**	**Taylor et al. (120)**
Age statistics (years)	Range	1.5–67	0–84	0–69[b]	0–54.3[b]	0–84	NA	NA
	Average	9.5	33.2	17.8[b]	7.7[b]	11.2	11.4	NA
	Median	7	35	12[b]	5.1[b]	NA	6.8	NA
Age groups	Pediatric (<5)	44 (37.0%)	86 (15.0%)	254 (31.2%)	900 (45.0%)	244 (48.8%)	NA	NA
	Pediatric (5–18)	63 (52.9%)	103 (17.9%)	266 (32.7%)	845 (42.3%)	175 (35.0%)	NA	NA
	Adult (>18)	12 (10.1%)	386 (67.1%)	294 (36.1%)	244 (12.2%)	81 (16.2%)	NA	NA
Sex	Males	67	220	453	1,101[c]	NA	NA	NA
	Females	52	355	361	888[c]	NA	NA	NA
Class tested	Probands	—	575	338	2,000	141	542	141
	Trios	119	—	410	—	338	2,081	6
	Other[d]	—	—	66	—	21	427	9
	Follow-up[e]	—	49	—	504	—	—	—
Did probands have prior diagnostic testing?		Yes	Most	Most	Some	Most	Most	Yes
Total cases		119	575	814	2,000	500	3,040	156

Abbreviations: NA, not available (data not provided in the publication); NCGENES, North Carolina Clinical Genomic Evaluation by Next-Generation Exome Sequencing. Dashes indicate that a particular class was not tested in the study.

[a] J.S. Berg, N.T. Strande, G. Haskell, B. Powell, A.K.M. Foreman, et al. unpublished data from manuscript in preparation.

[b] These numbers reflect demographic information only for individuals with a molecular diagnosis; information for other patients is not available.

[c] These numbers omit 11 fetuses with unknown gender.

[d] This category represents cases in which nontrio sets of family members were sequenced in addition to the proband.

[e] This category represents cases in which family members underwent Sanger sequencing of the candidate variants following exome sequencing in the proband.

Table 2

Diagnostic yields from seven large genome-scale studies

Study type		Exome (research study)		Exome (clinical laboratory)				Genome
Study		**Zhu et al. (136)**	**NCGENES[a]**	**Lee et al. (68)**	**Yang et al. (134)**	**Farwell et al. (39)**	**Retterer et al. (99)**	**Taylor et al. (120)**
Overall yield	Positive	29 (24.4%)	97 (16.9%)	213 (26.2%)	504 (25.2%)	152 (30.4%)	874 (28.8%)	33 (21.2%)
	Uncertain	0	119 (20.7%)	228 (28%)	NA	43 (8.6%)	1,575 (51.8%)	0
	Other[b]	21 (17.6%)	0	31 (3.8%)	NA	31/416 (7.5%)[c]	231 (7.6%)	23 (14.7%)
	Negative	69 (58.0%)	359 (62.4%)	342 (42.0%)	NA	215/416 (51.7%)[c]	359 (11.8%)	100 (64.1%)
Diagnostic Yield for each class	Probands	—	16.9% (97/575)[d]	21.9% (74/338)	25.2% (504/2,000)[d]	20.6% (14/68)	23.6% (128/542)	NA
	Trios	24.4% (29/119)	—	31.0% (127/410)	—	37.3% (82/220)	31.0% (647/2,088)	NA
	Other[e]	—	—	18.2% (12/66)	—	NA	NA	NA
Total cases		119	575	814	2,000	500	3,040	156

Abbreviations: NA, not available (data not provided in the publication); NCGENES, North Carolina Clinical Genomic Evaluation by Next-Generation Exome Sequencing. Dashes indicate that a particular category was not captured in the publication.

[a] J.S. Berg, N.T. Strande, G. Haskell, B. Powell, A.K.M. Foreman, et al. unpublished data from manuscript in preparation.

[b] This category includes candidate genes that were considered likely to be pathogenic.

[c] Eighty-four participants in this study opted not to have candidate gene testing and were thus not included in these numbers.

[d] Some individuals within these patient populations underwent follow-up testing to determine variant phase, segregation, and/or inheritance pattern.

[e] This category represents cases in which nontrio sets of family members were sequenced in addition to the proband.

Table 3

Phenotypic categories reported in seven large genome-scale studies

Study type	Exome (research study)		Exome (clinical laboratory)				Genome
Study	**Zhu et al. (136)**	**NCGENES[a]**	**Lee et al. (68)**	**Yang et al. (134)**	**Farwell et al. (39)**	**Retterer et al. (99)**	**Taylor et al. (120)**
Nonsyndromic ID	Undiagnosed genetic disorders	ID and Autism	DD DD and autism	Neurologic (?)	Neurologic	Autism Spectrum	#
Syndromic ID		Combination of: ID, dysmorphology, seizures, and other phenotypes	DD and hypotonia DD and epilepsy or seizures DD and dysmorphic features DD and heart disorder	Neurologic and other organ systems		Multiple congenital anomalies	
Neurologic		Neuromuscular disorders Ataxia Myopathy Leukodystrophy Seizures CNS	Ataxia/neurologic disorders Muscular dystrophy	Specific neurologic		Skeletal muscle Musculature Peripheral nervous Mitochondrial Seizures CNS	Ohtahara syndrome Other early−onset epilepsies
Metabolic		NA	NA	Nonneurologic	Metabolic/ biochemical	Metabolism	#
Sensory	NA	Hearing loss Retinal disorders	Retinal disorders		Ophthalmologic Audio-logic/ otolaryngologic	Hearing Vision	
Cancer		Hereditary cancer	Cancer predisposition		Oncologic	Neoplasm	Multiple adenoma
Cardiovascular		Cardiomyopathy Arrhythmia Aortopathy	Cardiomyopathy and arrhythmia		Cardiovascular	Cardiovascular	Hypertrophic Cardiomyopathy
Hematologic		Bleeding disorders	NA		Hematologic	Blood	#
Skeletal/ Connective Tissue		Skeletal dysplasia Connective tissue			Musculoskeletal/ structural Craniofacial	Connective tissue Skeletal (?) Growth (?)	Saethre−Chotzen syndrome (TWIST1 negative)
Immune		NA			Allergy/immunologic Infectious	Immune system	Common variable immunodeficiency disorder
Genitourinary					Genitourinary	Genitourinary	#
Dermatologic					Dermatologic	Skin	
Gastrointestinal					Gastrointestinal	Abdomen (?)	

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Study type	Exome (research study)		Exome (clinical laboratory)				Genome
Study	**Zhu et al. (136)**	**NCGENES[a]**	**Lee et al. (68)**	**Yang et al. (134)**	**Farwell et al. (39)**	**Retterer et al. (99)**	**Taylor et al. (120)**
Renal					Renal		Familial juvenile hyperuricaemic nephropathy
Liver					NA		Hemachromatosis
Pulmonary					Pulmonary	Respiratory System	#
Endocrine					Endocrine	Endocrine	
Other		Other			Dental Obstetric	NA	Systemic lupus erythematosus #

Only phenotypic categories that comprised more than five individuals are included. Abbreviations: CNS, central nervous system; DD, developmental delay; ID, intellectual disability; NA, not applicable; NCGENES, North Carolina Clinical Genomic Evaluation by Next-Generation Exome Sequencing. “(?)” indicates a lack in clarity regarding exactly which diseases are included in a particular described phenotype, and reflects our best guess as to what the category included. “#” indicates that phenotypes in particular categories were reported in the study but did not include more than five individuals.

[a] J.S. Berg, N.T. Strande, G. Haskell, B. Powell, A.K.M. Foreman, et al. manuscript in preparation.

Table 4

Sequencing details for seven large genome-scale studies

Study type	Exome (research study)		Exome (clinical laboratory)				Genome
Study	**Zhu et al. (136)**	**NCGENES[a]**	**Lee et al. (68)**	**Yang et al. (134)**	**Farwell et al. (39)**	**Retterer et al. (99)**	**Taylor et al. (120)**
Capture	Illumina TruSeq Exome Enrichment kit (65 Mb) Roche NimbleGen SeqCap EZ Human Exome Library kit Agilent SureSelect Human All Exon kit (50 Mb)	Agilent SureSelect^{XT}	Customized Agilent SureSelect Human All Exon V2 kit (50 Mb)	Roche NimbleGen SeqCap EZ HGSC VCRome (version 2.1)	Agilent SureSelect Target Enrichment System Roche NimbleGen SeqCap EZ HGSC VCRome (version 2.0)	Agilent SureSelect Human All Exon V4 kit (50 Mb)	None
Sequencing platform	Illumina HiSeq 2000	Illumina HiSeq 2000 and 2500	Illumina HiSeq 2000 (50 bp paired-end) or 2500 (100 bp paired-end)	Illumina Genome Analyzer IIx (100 bp paired-end) or HiSeq 2000	Not specified	Illumina HiSeq 2000 or 2500 (100 bp paired-end)	Illumina HiSeq 2000 or 2500 (100 bp)
Alignment software	BWA (version 0.5.10)	BWA	NovoAlign (version 2.07.15b) GATK (version 1.1-33)	BWA (version 0.5.10)	Not specified	BWA	BWA (version 0.5.6) Stampy (versions 1.0.12–1.0.22)
Variant caller	GATK (version 1.6-11) Annotation SnpEff (version 3.3)	GATK	VAX	Internal annotation databases	CASAVA Pindel	SAMtools (version 0.1.18)	Platypus (version 0.1.9)
Minimum coverage	10x	30x	10x	20x	10x	10x	22x
Variant filtering	Allele frequency, inheritance pattern, variant type, coverage, and quality score	Disease-based gene lists, allele frequency, variant type, coverage, and quality score	MySQL 5.2 database for filtering by variant type and MAF (analyzing variants with a MAF of <1%)	Cassandra (BCM software)	Stepwise filtering by variant type, MAF, and family history using the FIND bioinformatics program	Phenotype-driven gene lists (HPO and HGMD), inheritance patterns, variant type, phenotype, and population frequencies	Variant functional impact, frequency in the population, inheritance patterns and, statistical evidence for association (when applicable)

Abbreviations: bp, base pair; BWA, Burrows-Wheeler Aligner; CASAVA, Consensus Assessment of Sequence and Variation; FIND, Family History Inheritance-Based Detection; GATK, Genome Analysis Toolkit; HGMD, Human Gene Mutation Database; HPO, Human Phenotype Ontology; MAF, minor allele frequency; NA, not applicable; NCGENES, North Carolina Clinical Genomic Evaluation by Next-Generation Exome Sequencing; VAX, Variant Annotator X.

[a] J.S. Berg, N.T. Strande, G. Haskell, B. Powell, A.K.M. Foreman, et al., manuscript in preparation.

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