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An approach to pediatric exome and genome sequencing

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Abstract

Purpose of review—Exome and genome sequencing have recently emerged as clinical tools to resolve undiagnosed genetic conditions. Protocols are critically needed to identify proper patients for testing; select a test and laboratory; engage parents in shared decision making; and the return of results.

Recent findings—Among well-selected patients, the likelihood for identifying the causative gene change may be as high as 30%. It is key for pediatricians to consider whether sequencing should be the primary line of pursuit of a molecular diagnosis. Parents should understand the uncertainties inherent in this sequencing and the preference-based nature of testing. Pediatricians can engage in shared decision making for this process and work to help parents make decisions consistent with their priorities and values. Upon receipt of a pathogenic mutation, discussion of the likelihood for future treatment is paramount to parents, as are the implications for recurrence within the family. Uncertainties inherent to genomic results need to be explained in the context of the likelihood of future research and discoveries.

Summary—Pediatricians should make a deliberate decision with each patient whether to manage genomic testing on their own, refer the patient for such testing, or initiate the process and refer simultaneously. Regardless of which approach is taken, understanding the basics of this testing will allow the pediatrician to support the parents through the diagnostic process.

Keywords

genome sequencing; exome sequencing; informed consent; genetic counseling

INTRODUCTION

Genetic diseases are individually rare, but in aggregate common in pediatrics (1). More than 10% of pediatric hospitalizations are for children with a genetic disorder and genetic disease is a common cause of disability and death in pediatrics. The diagnosis of genetic disease is

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CONFLICTS OF INTEREST

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important – whether or not it leads directly to a specific treatment for the child it is essential to exclude treatable disorders, provide accurate recurrence risks for family members, assess prognosis, and end the diagnostic odyssey (2–4). Exome and genome sequencing (See Figure 1 for definitions) are changing our approach to the diagnosis of genetic disease and it has advanced rapidly from a theoretical possibility just a few years ago (5) to a test that a clinician can order (6–9). Successful integration of exome and genome sequencing into pediatric care will include helping parents to have realistic expectations of this new technology. Although testing can be successful in identifying a genetic cause, in the majority of cases a cause will not be identified. Yet over time a cause may be identified as novel variants are identified and interpreted. This review describes important attributes and considerations for diagnostic genome and exome sequencing in the pediatric setting.

TESTING CONSIDERATIONS

A key, early decision that the pediatrician must make is how much of this process they wish to engage with. Pediatricians often use single gene testing (which uses Sanger sequencing technology) and genomic sequencing merely broadens the scope of what can be identified while decisions to use it parallel older methods for identifying the etiology of mendelian genetic disorders. For example, if the pediatrician is comfortable with a thorough workup for developmental delay, adding exome or genome sequencing to that process is something they can incorporate into their practice. On the other hand, for patients with rare, complex malformation syndromes, it may be more efficient to refer the patient to a clinical geneticist early and work with the genetics team and leave the sequencing and other management challenges to subspecialists. There is a middle road as well – if the indication for sequencing is sound, the pediatrician can concomitantly order the sequencing and initiate the referral to the geneticist - the patient can arrive in the genetics clinic with a sequencing result, saving months of time. Our objective in this review is to help the pediatrician decide how much of this testing to do themselves versus referring the patient to subspecialists.

Key attributes of genome and exome sequencing

A key concept to grasp with genome and exome sequencing is that they evaluate ~20,000 genes, but don't evaluate all genes. Unfortunately, they are often described as “whole genome sequencing” or “whole exome sequencing”. The use of the word “whole” distinguishes them from single gene or gene panel tests, but use of the word “whole” may mislead clinicians or patients to conclude that it is 100% sensitive for detecting disease causing mutations. In fact, for a number of reasons (Figure 2), the coverage of known genes by these tests is 85–92%. These averages encompass some genes that have very low coverage and many genes that have ~100% coverage.

Choosing single gene/gene panel versus genome/exome sequencing

That the coverage of genome and exome sequencing is <100% is a key factor in determining whether a patient should undergo such sequencing versus a gene-specific test or multi-gene test panel. As noted above, a key question is whether a single gene or gene panel test should be ordered versus a genome or exome sequence. This is dependent on the patient and also affected by the ability of the ordering clinician to recognize specific disorders or syndromes.

For patients with nonspecific manifestations (e.g., intellectual disability without other recognizable distinguishing features) genome and exome sequencing can be clinically indicated. In contrast, a patient with intellectual disability and a number of other specific dysmorphic features may have a recognizable syndrome that would allow a clinician to order a single gene test (e.g., Rett syndrome). This is even more important for disorders that are caused by mutations that cannot be detected by current genome and exome sequencing. For example, Fragile X syndrome includes intellectual disability and has other features that can be recognized by an experienced clinician. The typical mutation of that disorder (expansion of a three nucleotide repetitive DNA sequence) is not detectable by current genome and exome sequencing. On the other hand, many patients undergo a diagnostic odyssey where a series of single gene tests are used to search for a diagnosis. This can be wasteful because three to six single gene tests can cost as much as an exome or genome test. Finally, there is the question of exome versus genome sequencing. Currently, genome sequencing costs are two to three times that of exome sequencing. Most identifiable single gene mutations that cause recognizable human genetic disease are in exons (and 85–92% of those would be identified in an exome sequence result). But genome coverage can include regions poorly covered by exome sequence. Over time, exome sequencing will be displaced by genome sequencing as more non-exonic disease associated mutations are described. For a given patient, the ordering clinician should consider discussing the clinical scenario with the genome and exome sequencing laboratories to determine which approach is preferable. Third party payer considerations may also play a role here.

Tests that should be considered prior to genome/exome sequencing

Clinicians also need to consider tests other than sequencing to evaluate their patients. For example, 5–15% of patients with intellectual disability have large segments of DNA that are duplicated or deleted (copy number variants). These lesions are not well evaluated by genome, exome, gene-specific, or multi-gene panel sequencing and instead should be assessed by array testing (10). As well, there are literally thousands of metabolite, enzyme assay, imaging, and other diagnostic techniques that can be used to narrow the differential diagnosis for a patient.

Determining the stage of evaluation where exome/genome sequencing is appropriate

The key is to decide for a given patient how far to go in refining the phenotype assessment to identify a specific diagnosis that could point to a single gene or panel test, order an exome or genome sequence, or refer the patient to a genetic specialist for further evaluation. This is an emerging area and there are little data to identify the most efficient and effective path. The pediatrician should use their training and experience to consider the patient characteristics and apply their clinical judgment as which approach seems most appropriate. The evaluation of most patients will warrant a substantial amount of non-DNA testing prior to sequencing, but it is important to distinguish those who will not benefit from this approach from those for whom sequencing is the most efficient path forward. Although genomic sequencing tests are expensive, it is not rare to spend much larger amounts of testing dollars fruitlessly searching for a specific diagnosis when an exome or genome could readily identify the causative mutation for less money (11). Genome and exome sequencing suggest diagnoses for patients with atypical presentations that elude clinical diagnosis (12).

Again, because the technology sequences essentially all genes, a variant can be identified in a gene not previously considered in the differential diagnosis. When that variant is identified, the clinician can re-evaluate the patient for manifestations and symptoms of the disorder suggested by the variant and determine if the symptoms match sufficiently to confirm the diagnosis.

Who to sequence

In the pediatric clinic setting, the most common presentation is that of a single affected child in a family. In these cases, it is appropriate to sample both parents and the child. Some laboratories sequence all three samples, others only sequence the child and do reflex testing for specific variants identified in the child on the parental samples (to validate variants and assess inheritance). In families with multiple affected individuals, the decision regarding who to sample is more complex and is addressed elsewhere (13). Commonly, multiply affected families are evaluated by geneticists.

Choosing the testing laboratory

Sequencing laboratories vary in their methodologies, in how they conduct sequencing and report out results. Comparing options can be daunting and many providers select the lab based on the likelihood that the parents' insurance or medical assistance will reimburse for the testing or whether their institution has a contractual relationship with a certain laboratory. Third party payers have heterogeneous policies regarding reimbursement of genome sequencing and genetic counselors can be contacted for consultation on the relative differences. One consideration is whether the lab has a policy to return to the sample for re-sequencing or re-interrogation if the initial interpretation is negative.

PRE-TEST CONSIDERATIONS

Once genome or exome sequencing have been identified as appropriate for a patient, the parents should be engaged in a decision making process. Undergoing sequencing may not yield the sought after information, can result in unexpected health information (14), or may yield uncertain information. As such, decisions to sequence one's child can be described as preference-based, in contrast to medically indicated. Preference-based decisions lend themselves to shared decision making (15, 16). Shared decision making refers to value based decisions for which there are two or more reasonable options (17). In the case of genome sequencing, given the yield and limited clinical utility, reasonable parents may decide not to undertake sequencing. After learning more about the complexities and uncertainties, some parents may decide that they do not wish to continue on an odyssey in pursuit of a diagnosis or etiology for their child's condition.

That unexpected health information can be generated is a consequence of the fact that exome and genome sequencing are broad evaluations of thousands of genes, and there is a chance that a genetic susceptibility to a condition unrelated to the indication for the test could be identified. These are described as secondary or incidental findings. Current recommendations are to include analysis of a prescribed and relatively small set of genes

(currently 56 genes) and it is the default to perform this evaluation, with an opt-out mechanism.

Pre-Test Counseling

Shared decision making about sequencing should include elements central to sequencing. These include the various sources of uncertainty: ambiguity, likelihood, and complexity that relate to interpreting pathogenicity, penetrance, and future health risks (18, 19). Importantly, the discussion should include the meaning of a "negative" result that differs for sequencing compared to other medical testing. A variant may have been overlooked or missed given the state of the science and as such cannot be interpreted as reassuring or a "clean bill of future health." Further, to help establish realistic expectations, parents should be engaged in a dialogue about potential treatment or cure. While many parents will express reasonable expectations, acknowledging the limitations of the science, they simultaneously hope for useful information for their child's future care (20). Shared decision making can reveal parental motivations for pursuing sequencing in their child and foster understanding of related values and beliefs that can allow pediatricians to helping parents choose the course of action most consistent with their values and priorities.

Informed Consent

Upon selection of a lab, there will be a consent form that acknowledges an understanding of the relative risks and benefits of sequencing. Consent forms can be lengthy, highly technical, and written at a high literacy level. Rather than ask parents to navigate the forms on their own, we recommend the shared decision making approach described above. The pediatrician can make a checklist of the essential points in the consent form to ensure that the content is included in the discussion in light of parent priorities. Genetic counselors may also be involved to engage parents in making an informed choice whether to undergo sequencing (21, 22). Some testing laboratories offer telephone counseling, and many universities offer in person genetic counseling services that would be appropriate for this counseling.

POSTTEST CONSIDERATIONS

A key to approaching exome and genome sequencing results is that they are not "positive/negative" assays. They can be better considered as broad surveys for potential genetic mutations - which is both the power and the weakness of the tests. They are powerful because they can identify the cause of the disease in a patient without the need to select the correct single gene test (23). The weakness is that the multiple testing problem –a typical exome sequence result includes 30,000–40,000 variants and zero to two of those variants are the cause of the disorder (zero if the causative mutation(s) are not identified, one if the cause of the disorder is identified and it is due to a single mutation or two if it is caused by two mutations in one gene). The key to interpreting genome and exome sequencing is to distinguish the few true positives from the large number of false positives. Whenever the ratio of true positives to false positives is small, the risk that latter may be mistaken for the former is high.

Interpreting genome and exome findings

The clinical laboratory will attempt to identify the causative variant from all those generated by the test in the context of the clinical information supplied by the ordering clinician. The clinical laboratory may conclude that they have confidently identified a causative mutation. Even though the test is considered to be positive, the ordering clinician, in concert with their consulting colleagues, must confirm that the result makes sense in light of the patient manifestations. Overall, exome and genome sequencing yields a positive result in 20–30% of patients. In other cases, the clinical laboratory may determine that they have identified one or several variants that are possible causes of the manifestations of the patient. This can generate both confusion and engender a great deal of subsequent clinical investigation to determine if one of these variants is indeed causative. One of the key actions here is for the clinician to review the candidate variants and perform follow up evaluations that might distinguish among them. This activity turns clinical practice on its head – the genetic test results drive phenotypic evaluations, instead of the classic paradigm where the phenotypic evaluations determine which genetic test is ordered (23). As well, this clinical activity may extend beyond clinical care into clinical research activities if the variants are in genes that are not firmly associated with human disease. Here the pediatrician should engage with subspecialists – typically clinical geneticists who are familiar with genomic testing. As well, the patient may need to be considered for enrollment in a clinical research study. Finally, the test result may be negative – the clinical laboratory may identify no variants that are plausibly related to the disorder in the patient. This result should prompt a re-evaluation of the theory that the disorder is indeed genetic. Other causes, such as teratogenic (e.g., fetal alcohol syndrome) should be considered. The exome or genome test may be negative because the current state of research has not identified the gene that has been associated with the disorder that is affecting the patient. Future research may identify this gene, but it is unclear how previously performed exome and genome sequencing results should be re-evaluated to take future research discoveries into account.

Return of results to the patient's parents

Once an interpretation of a variant is made, the results should be communicated to the parents. Regardless of the pre-test counseling and consent process, parents of a child with an undiagnosed condition likely harbor high expectations for the results. Discussion of the gene and how the variant is thought to cause the condition can be meaningful to parents as a long sought after answer. From there, parents will be concerned with potential health implications for their child. For novel variants, the expressivity of the disorder may be unknown. The potential for additional manifestations of the disorder to appear over time should be discussed. As well, parents will want to understand the potential for treatment based on identification of a pathogenic variant. While no treatments are likely to be imminent, one can convey to parents that identification of the cause is an important first step. On rare occasions, more often for metabolic conditions, treatment of the disease process may be on the horizon. Throughout this discussion, the uncertainties inherent in the information should be conveyed. At times there will be relative certainty, for example even when there is ample evidence for pathogenicity, the gene may be newly recognized and thus treatments unavailable. Often results will be ambiguous, such as variants of unknown clinical significance. Since it is atypical in non-genetic clinical practice to return a result of such

questionable clinical utility, parents will need to be helped to understand the sources of the uncertainty and an estimate of the likelihood that it may be resolved. Parents may question the trustworthiness of sequencing results in the face of such ambiguity (18). Parents may benefit from understanding that interrogating a genome often yields incompletely understood findings that are pre-clinical in their usefulness. Counseling may be valuable for managing the uncertainty and fostering realistic expectations of future clinical clarity.

Mode of inheritance

Identification of a variant(s) may identify the mode of inheritance and thus risks to the parents of recurrence in a subsequent child and risk to their relatives. This is achieved by testing the parents for the presence of the variant(s) found in their child (for details see (13)). Understanding the mode of inheritance can provide parents options for family planning and for helping relatives understand their risks. Some parents seek this information as their primary motivation to pursue genomic sequencing.

Parental expectations for sequencing

Parents of affected children and adult patients often find personal utility in sequence information, even when clinical utility remains elusive (24, 25). Parents of children with undiagnosed conditions report the importance of learning any information (26). Knowing is perceived to be better than living with the lack of any information. This may be viewed as replacing one uncertainty with another, yet parents often value any information as momentum toward future understanding. Responses to uncertainty relate to prior epistemological beliefs and set parental expectations (18). For parents struggling to manage the uncertainties, referral to a genetic counselor may be prudent.

SUMMARY AND CONCLUSIONS

Clinical exome and genome sequencing are powerful tools to identify the genetic cause of heritable disorders in children. While it may be appropriate to refer candidate patients for exome or genome sequencing to subspecialists, the motivated pediatrician can incorporate this testing into their practices as a part of a well-organized evaluation for common presentations such as non-syndromic developmental delay or intellectual disability. An understanding of the pretest, testing, and posttest considerations can allow the pediatrician to participate in this process and help guide their patients and their families into this new era of genomic medicine.

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KEY POINTS

- Pediatricians can participate in the ordering, interpretation, and down steam use of exome and genome sequencing
- The core attributes of this testing are that it is at once a broad genetic test (~20,000 genes) but does not evaluate 100% of genes
- These core attributes determine that the test can have false positives and is not 100% sensitive, but it can diagnose 20–30% of patients with genetic disorders
- Other tests, such as chromosomal microarrays or metabolic testing, should precede exome or genome sequencing of selected patients
- Pre-test and post-test counseling issues can be mastered by the pediatrician and become a critical part of guiding families through this testing process

- Coverage: The fraction of the gene that is adequately interrogated by the sequencing process
- Exome: The DNA of the nuclear genome that encodes the exons of genes.
- Exons: The portions of genes that are spliced together to form the messenger RNA. This comprises 1-2% of the genome
- Expressivity: The variance in manifestations of the disorder among affected patients
- Genome: The entirety of the DNA of the nuclear genome. Note that this definition does not include the mitochondrial genome
- Sanger sequencing: A well-established method that uses polymerase chain reaction to amplify a portion of DNA, followed by another amplification reaction that uses special DNA nucleotides to stop the sequencing reaction and the molecules are separated in a gel matrix and imaged with a laser. This technology sequences one portion of DNA at a time.
- Variant: a sequence result that differs from the human reference sequence

Figure 1. Key Definitions and Terms

There are a few key terms that pediatricians may be unfamiliar with that are important for discussing genomic testing.

- Not all genes that when mutated in humans cause disease have been identified
- Sequencing coverage is less than 100%
- The DNA sequencing chemistry and instruments cannot sequence some segments of DNA (e.g., repetitive DNA like the Fragile X repeat expansion)
- The human genome reference sequence (to which patient DNA sequence has to be aligned to be interpreted) is incomplete and has errors
- Some types of mutations are poorly detected by genome and exome sequencing (e.g., large deletions)

Figure 2. Some reasons why Genome and Exome sequencing does not evaluate all potential disease-causing mutations

A key concept of exome and genome sequencing is that it does not assess all types of DNA alterations that can cause human disease. Knowing some of these limitations is important for considering whether the diagnostic approach to the patient should include this testing.