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EDITORIAL

Inconclusive results in genetic testing: explaining uncertainty

Testes genéticos inconclusivos: Como explicar a incerteza

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Genetic testing, particularly exome sequencing (and soon genome-wide sequencing), has become widely available as a diagnostic tool in the field of pediatrics. This advancement has increased the diagnosis of underlying genetic conditions while reducing the need for invasive procedures, particularly muscle biopsies in neuromuscular diseases.[1] For instance, whole exome sequencing (WES) has now an overall diagnostic yield ranging from 20% to 50%, depending on clinical suspicion, slightly lower than the one reported for whole genome sequencing (WGS).[2] As new associations between genes and disorders continue to emerge and the compilation of information on rare findings increases, the diagnostic yields will rise even further.

However, with greater sequencing capacity comes a greater number of candidate variants that need clinical significance assessment. Relevant findings may be overlooked, while on the other hand, a significant amount of information unrelated to phenotype can find its way into the clinical report. Since it is up to the clinicians to communicate the genetic testing results, it is their responsibility to assess the report's content and to delineate the best way to inform the patient/family.

The American College of Medical Genetics (ACMG), in collaboration with other scientific associations, has been providing recommendations for variant classification since 2000.[3-5] Based on guidelines from the ACMG, genetic variants can be classified into five different categories: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign or benign.[3] A VUS is a genetic variant with unclear implications on gene function. Unlike pathogenic and likely pathogenic variants, the relevance of theses VUS is unknown due to lack of scientific evidence to determine wether it is clinically causal, and potentially actionable, or benign. However, a VUS classification may change through additional research, namely familial studies or other complementary assays, or new scientific and validated evidence. Thus, it is not uncommon for a variant initially believed to cause a disorder to later be reclassified as benign, and the reverse scenario has been documented too. This underlines the need for caution while conveying genetic information.

A new ACMG classification system is expected to be published by the end of 2023.[6] While it is likely to alter, to some extent, the way VUS are classified, it is expected to maintain categories for variants that may or may not be indicative of a diagnosis.

VUS are highly prevalent in reports and pose significant challenges for management in the clinical setting. In a retrospective study by Cornthwaite et al, focusing on prenatal WES for fetal anomalies, 42% of studies reported VUS.[7] Among these, the vast majority were found in genes that turned out to be irrelevant to the phenotype, although around 5% were subsequently recognized as diagnostic markers. In the author's own cases involving sequencing studies, regardless of the clinical features, about one-third of variants in relevant genes remain classified as VUS. While variants entirely unrelated to the phenotype are often excluded from clinical reports, the degree to which results are filtered out varies based on laboratory practices.

Functionally, VUS are generally either benign or pathogenic. However, given their nature and frequency, a definitive determination of their impact is a hard task. The subsequent analysis of these variants and the potential approaches for reclassification (including enzymatic studies, functional assessments, segregation studies, and collaboration with other patients and research groups) form a

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substantial part of a clinical geneticist's work today. Still, a significant proportion of these variants are likely to remain as VUS.

Parents, guardians, or even older children are entitled to receive test results relevant to the child's condition. Often, they hold a binary expectation of how test results will turn out. While no comparative studies are available, there is a notion that anxiety related to chronic illnesses could be more pronounced in pediatric settings, especially among parents. The mere presence of a variant in a gene linked to a disorder, regardless of likelihood of being deleterious, tends to trigger inquiries and worries. The concept of a VUS is challenging for patients to grasp and for physicians to explain. The unexplained existence of a VUS might lead caregivers to doubt the accuracy of a diagnosis, generating unnecessary anxiety, especially if the associated prognosis is unfavorable. This uncertainty might even prompt parents to seek irrelevant treatments for their child or foster feelings of guilt when the variant is inherited. Discrepancies in the interpretation of findings by different physicians or alterations in classification can further perplex the comprehension of the conveyed information.

Even with a comprehensive explanation and counseling, the uncertainty surrounding the impact of a variant in a pediatric context can give rise to concerns and frustrations among the child, their parents, and their physician. These concerns are valid because inconclusive results can introduce doubt without truly providing actionable diagnostic information.

Prior to conducting the test, it is beneficial to inform parents and older children about the possibility of encountering VUS, and what that would mean for patient care. Additionally, they should be made aware that variant classification might change over time and what will be the strategy of management and follow-up in that setting. This proactive approach should facilitate the communication of results.

Generally, patients and families can comprehend that undergoing the study, even if inconclusive, yields more insight than not undergoing it at all. Also, confirming the steps that will be taken to potentially reclassify the variant, and if it closely aligns with the patient's phenotype, the monitoring for possible complications associated with the disease, can reduce the psychological distress and the diagnosis-specific concerns.

Ideally, patients and families dealing with a VUS should engage in genetic counseling through a geneticist to discuss the variant's implications, the meaning of this finding and medical management recommendations based on the results. Some studies suggest that parents seem to become more comfortable when provided with more information, especially in the written form. This is a challenging task for the geneticist. Besides information, it is equally important to understand patients' priorities and needs and to develop a shared understanding, a patient-physician relationship based on trust and confidence to support the children and families to allow an informed decision-making process. For certain families, psychological or psychiatric support might be necessary and should be considered.[8-10]

On the other hand the non-genetics healthcare professionals, that expect to use the evidence of the genetic testing in medical management decisions, find difficulties in the interpretation of these results.[11] Consequently the importance of cooperation of geneticists with pediatricians is essential, explaining in general what a VUS is and what it might mean specifically in the patient's context considering the patient's medical and familial history and the strategy for follow-up .

Despite their undeniable utility and potential for diagnosis, WES and WGS tests do not always meet the expectations of patients and families, especially in a pediatric setting. They should understand that the finding of a VUS in a child with a distinct phenotype cannot be determined to be causative of the condition and therefore predictive testing will not be possible for at-risk family members at that time. Appropriate preparation, counselling, and ongoing support should be put in place to overcome the challenges of conveying high-throughput genetic testing results.

The rapid developments of genetic technologies impose a strong and effective communication and collaboration between pediatricians and geneticists in order to ensure continuity and improvement of patient care and genetic literacy.

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