The scary world of variants of uncertain significance (VUS): A hitchhiker’s guide to interpretation

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This short review will focus on how to interpret variants of uncertain significance (VUS) in the setting of newborn screening (NBS) for severe combined immunodeficiency (SCID). Many of the tenets of this review can be extrapolated to other settings; however, NBS for SCID represents a best-case scenario for variant interpretation because nearly all the genes are known and because the phenotype is easily defined, usually as a T-cell count. The interpretation of gene variants is rapidly evolving, with individual variants being reclassified regularly as new data become available.1-3 Therefore, this review will start with the current nomenclature schema, recognizing that classification of variants is not fixed. A fairly unique aspect in immunology is that gain-of-function (GOF) variants are increasingly associated with disease and they are portrayed in this figure although loss-of-function (LOF) variants are more often encountered in the setting of SCID (Fig 1,A).4 GOF refers to a protein with increased function, even if the increased protein function is deleterious to the cell. In contrast, LOF refers to a protein with compromised function. Protein function is the aspect that participates in cell biology; however, sequence results do not directly report the effect of the variant on protein function in most cases because it is unknown. Sequencing reports categorize a sequence variant into likelihood of altered protein function. The American College of Medical Genetics has recommended a 5-tier system for classifying variants,5 and this terminology is used on most sequencing reports although the methods used for classification can differ (Fig 1, B).

Reporting of a VUS can happen with Sanger sequencing for a single gene, a gene panel, whole-exome sequencing, or whole-genome sequencing.6 Variants are reported in the same way regardless of the technique used. Fig 2 represents an approach to understanding next steps when a sequencing report arrives for an infant with presumed SCID. In some cases where a narrow range of genes was assayed, the algorithm will suggest expanding the sequencing approach. This refers to moving from sequencing a smaller number of genes to a larger number of genes and considering copy number variation. The algorithm may also suggest phenotype matching and in the setting of NBS SCID7 patients, this refers to a low T-cell count or a low CD4/CD45RA count along with any syndromic features. Finally, determining whether the variant segregates in the pedigree refer to determining whether the variant in question is found in any other affected children. The algorithm references a key step for autosomal-recessive (AR) conditions and that is the determination of whether each of the 2 variants are found on separate chromosomes, referred to as separate alleles. Generally, one allele on one chromosome is inherited from the father and the other allele on the second chromosome is inherited from the mother. There can be 2 variants identified but if they are on the same allele from 1 parent, then there is potentially 1 gene that is still classified as normal from the other parent. There may be a hidden variant on the second allele but until identified cannot be presumed. The situation is quite different for X-linked recessive disorders such as SCID due to deleterious mutations in IL2RG. Here, a single variant can cause disease but a VUS must not be assumed to cause disease. Similarly, for an autosomal-dominant condition such as RAC2 GOF mutations associated with SCID, a single pathogenic variant is sufficient for disease; however, a VUS must not be assumed to be causal.

In about 10% of cases where sequencing is performed for SCID, there may be no variants reported. Awaiting a genetic diagnosis is not necessary to move toward therapy. There are 3 main approaches for this very frustrating circumstance: (1) escalate to more expansive sequencing, (2) perform copy number variation analysis, or (3) consider a syndromic diagnosis not necessarily associated with a genetic basis.

These workflows all use Clinical Laboratory Improvement Amendments (CLIA)-approved studies and do not invoke any research approaches. If there is a VUS in a gene known to cause SCID but not previously reported as pathogenic, it could be useful to try to garner additional data to support its assignment as possibly causal. Rarely, a gene not previously reported to be associated with SCID is identified. There are research approaches that can be helpful in these circumstances. Several of these approaches are quite easy:

1. Matchmaker approach to see whether there are similar patients found by others
Clinical Immunology Society VUS listserve
Matchmakerexchange.org

2. Check Gnomad for other variants at the same amino acid.
Check how tolerant the gene is of LOF variants. Two scores are reported: LOF observed/expected. Low observed/expected scores (<0.5) indicate low tolerance for LOF. pLI is the second score. High scores (>0.9) indicate low tolerance for LOF.
Gnomad.broadinstitute.org
FIG 1. Variant pathogenicity and variant classification. A, The y-axis indicates the degree of dysfunction for either GOF or LOF of the variant. Less function to the left of midline indicates LOF alleles, whereas increased function is indicated to the right of midline as GOF alleles. RAC2 GOF mutations are the only commonly identified GOF variants identified on sequencing for SCID. In the setting of SCID, there are individual variants in genes leading to fully penetrant SCID and those that lead to hypomorphic SCID. There are surely variants with even lesser effects on T-cell development that might lead to very mild T-cell lymphopenia that have yet to be cataloged. B, Variants are classified into 5 categories on a range from benign to pathogenic on the basis of bioinformatic approaches and available clinical data. This nomenclature appears on most sequencing reports.

FIG 2. Workflow for VUS identified in infants with low T cells. The pathway on the left is provided as a comparator. It represents the ideal scenario where 1 (for X-linked recessive or autosomal-dominant conditions) or 2 (for AR conditions) pathogenic variants are identified. The 3 pathways where VUS are identified by sequencing lay out strategies that can lead to assignment as probably causal. CNV, Copy number variation.
3. Look up the variant in Catalogue of Somatic Mutations in Cancer (COSMIC). Highly enriched variants in cancer imply altered function. Cancer.sanger.ac.uk/cosmic

Of much greater difficulty is defining the functional consequences of a specific variant in vitro. If there is a laboratory with a specific interest, it may have data or may be willing to make an expression vector for the variant. This is a significant effort on the part of the laboratory, and it is important to recognize that a single assay for function may not fully reflect the cellular role of the protein. The degree of difficulty is even higher with the production of an animal model. These require interest, expertise, and dedicated funding.

Moving into this new era of sequence-based approaches to diagnosis requires new skills with nomenclature and technology but can lead to improved diagnosis and management.9,10

There are a few cautions to bear in mind when assessing VUS pathogenicity.

CAUTIONS

- Common variants in isolated populations may not be cataloged in large databases.
- Variant pathogenicity classifications change over time, both toward more pathogenic and less pathogenic.
- Do not accept a VUS as causal just because it makes sense.
- For AR conditions, ensure that the variants are on different alleles.
- Be very wary of heterozygous VUS in genes where the inheritance is usually AR unless there is a pathogenic variant on the other allele.
- Copy number variation accounts for 5% to 10% of all deleterious genetic effects.

This short review provides an overview of approaches for clinicians faced with a VUS in an infant with low T cells. Similar but more complex approaches are needed for other conditions where the phenotype may be much broader, have no consistent laboratory phenotype, or have variable penetrance. There are ongoing efforts to improve the foundational information to allow improved analysis of variants and this conundrum will fortunately become less frequent over time.

In grateful acknowledgment of the many lessons from patients and colleagues.

REFERENCES