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Variant of Uncertain Significance Identified in Exome Sequencing: What Next?

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Abstract

Next-generation sequencing (NGS) has revolutionized the field of medicine, providing better tools for the diagnosis of genetic diseases. However, it has also thrown up new challenges like reporting of variants of uncertain significance (VUSs). All clinicians who order for a test based on NGS are likely to come across this entity. This article is an attempt to guide the clinician regarding the further steps to be planned once a VUS is identified in their patients.

Keywords: Next-generation sequencing (NGS), variant of uncertain significance (VUS), segregation, reverse phenotyping.

Introduction

Next-generation sequencing (NGS) is a technique of massively parallel DNA sequencing which is useful for sequencing of multiple genes or whole exome/ genome for finding a genetic etiology in the patient. NGS technologies are not only cost effective and rapid, but also highly accurate and reproducible. NGS is majorly used in clinical practice for diagnostic evaluation to identify a genetic etiology of disease in the patient which helps in early diagnosis and proper management of the disease as well as for better understanding of disease mechanisms. Currently different NGS approaches being used are targeted panels sequencing (TPS), whole-exome sequencing (WES)/ clinical exome sequencing (CES) and whole-genome sequencing (WGS). TPS is used for diseases/phenotypes which are known to be caused by a group of genes e.g.,genetic epilepsy panel, muscular dystrophy panel, etc. CES is a large panel of about 5500 genes wherein sequencing of genes known to cause single gene disorders in humans is done. WES is sequencing of all ~20,000 genes in the human genome. WGS refers to sequencing of the entire genome including the coding and non-coding regions.

Whole-exome sequencing (WES) is sequencing of entire coding regions of the genome (exons of ~20,000 genes), which constitutes ~1% of the genome. It is known that 85% of Mendelian disorders are caused by pathogenic variants in protein-coding regions. Hence WES is likely to give the genetic diagnosis in a large proportion of patients. WES is widely used in patients with a suspected Mendelian disorder where a definitive diagnosis is not available e.g., syndromic intellectual disability, multiple malformation syndrome, etc. (Bamshad et al., 2011). It is cost effective compared to WGS with a diagnostic yield of ~15–45%. The major disadvantages of WES include inability to identify variants in non-coding regions of genome (introns, intergenic regions) and to detect different genetic mechanisms like copy number variations, triplet repeat disorders, methylation disorders, etc. (Bhowmik & Dalal, 2017).

It is very important that every clinician should be aware regarding interpretation of an NGS report since NGS-based testing is being routinely used in clinical practice. It is easy to interpret the report if a systematic method is followed. An NGS report mainly comprises of following segments:

1. **Variant information:** Gene name, transcript ID (most abundant mRNA of the gene), location of the variant – chromosome number, nucleotide position on the chromosome, position on mRNA (c.) and position on protein (p.), zygosity (presence in homozygous or heterozygous form in the patient).
2. **Disease information:** Name of the disease, OMIM details, segregation pattern of the disease
3. **Variant frequency information:** indicates presence of the variant in public databases like 1000 Genomes, gnomAD etc. with minor allele frequency (MAF), or in mutation databases for known variants like ClinVar, OMIM, etc.
4. **Pathogenicity information:** indicates pathogenicity prediction by various tools for novel variants.

Based on the above information, the classification of variants is done using the American College of

Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/ AMP) guidelines, that classify variants into 5 classes i.e., pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign and benign (Richards et al., 2015). A variant is classified as a pathogenic or likely pathogenic variant, if the variant is found in a gene previously described with clinical features of the disease in the patient and is reported at very low frequency in the normal population. The variant is classified as benign/ likely benign if the variant is present in the normal population at a high frequency and is not predicted to be damaging for the protein. However, if we are not able to confidently classify a variant as pathogenic/ likely pathogenic or as benign/ likely benign, then the variant is classified as a variant of uncertain significance (VUS). VUSs mainly include novel variants in genes related to the phenotype in the patient which are found at very low frequency in the normal population. VUS in NGS reports should be interpreted with caution by clinicians, and the same should be explained to patients. VUS should not be used for irreversible actions like genetic counseling, prenatal diagnosis or carrier screening.

What next?

Reclassification of VUS to pathogenic/ likely pathogenic or benign/ likely benign can be done as more evidence is obtained regarding the variant. The steps to be followed after finding a VUS are (a) check the Mendelian segregation of the variant in the family;(b) do reverse phenotyping; and (c) follow up the patient for any future reclassification of the variant.

(a) Mendelian segregation of the variant in the family

Analysis of Mendelian segregation of the variant can help in obtaining further evidence towards pathogenicity of variant **(Figure 1)**. In cases of a heterozygous VUS in a gene known to be associated with an autosomal dominant condition, Mendelian segregation can show the same variant in one of the parents or both parents may be homozygous for the wild type allele. If one of the phenotypically normal parents is carrier of the heterozygous variant, then the variant is less likely to be disease-causing. However, possibility of incomplete penetrance needs to be considered **(Figure 1A)**. If the variant is of de novo occurrence in the patient, as is evident by homozygous wild type allele in both parents, then the variant is likely to be disease-causing and needs further analysis for functional significance. Similarly, if the variant is homozygous in the proband and the gene is known to be associated with an autosomal recessive disorder, then there are three possibilities in segregation analysis **(Figure 1B)**:

A. If both parents are found to be heterozygous carriers for the same variant, then it points towards autosomal recessive inheritance of the variant.

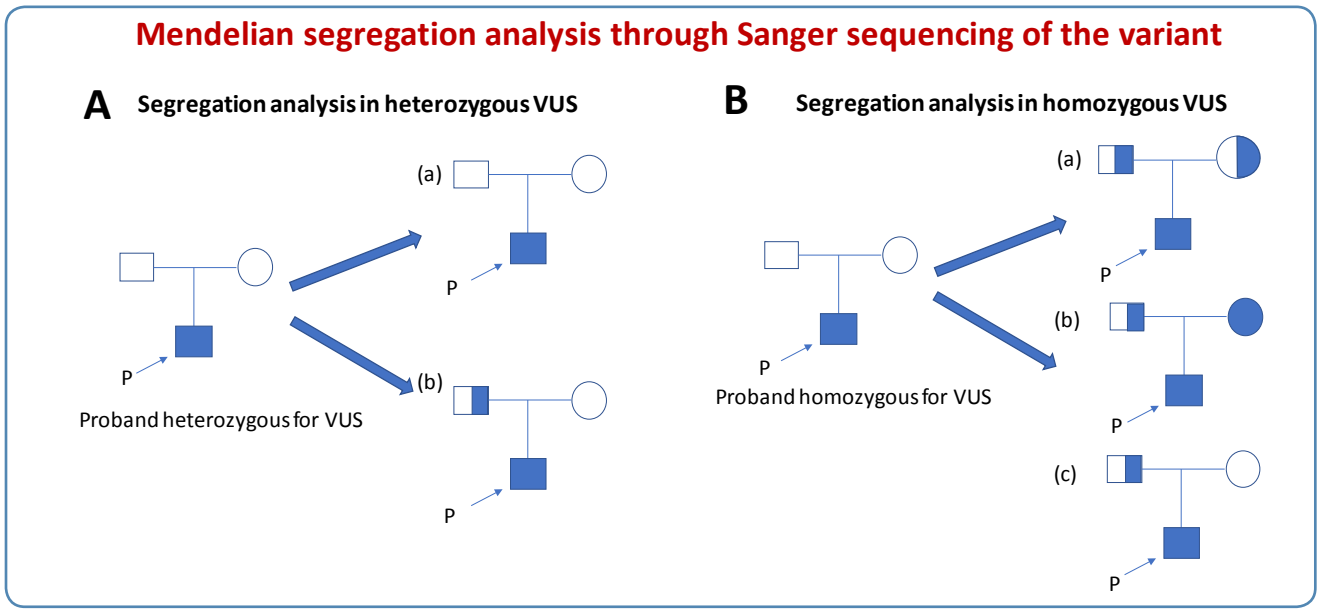

Figure 1 Utility of Mendelian segregation analysis for variant interpretation

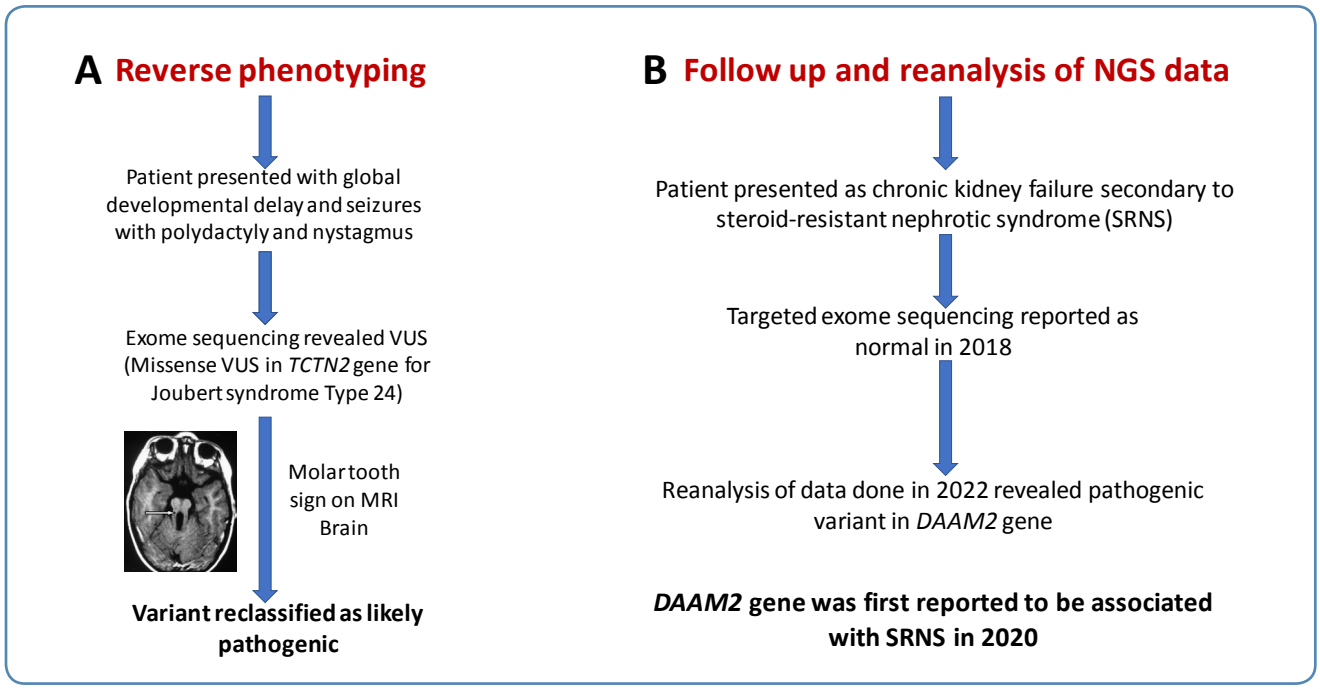

Figure 2 Examples of reverse phenotyping and data reanalysis for variant interpretation

B. If one of the phenotypically normal parents is homozygous for the same variant, then such a variant is less likely to be pathogenic.
C. If one parent is found to be a heterozygous carrier and other parent is homozygous wild type for the variant then possibilities of large deletion not detectable on Sanger sequencing, uniparental disomy and disputed paternity need to be considered.

(b) Reverse phenotyping

A VUS can be further refined and reclassified using additional evidence based on functional assay or identification of specific radiological/biochemical evidence for a particular disease. Functional evidence can be in the form of investigations like enzyme assay, immunohistochemistry on muscle biopsy, magnetic resonance imaging studies,etc. **(Table 1 & Figure 2 A)**. Reverse phenotyping is very important in present times since many of the genetic tests are done using a genotype-first approach without obtaining sufficient radiological/ biochemical/pathological clues for diagnosis of the condition.

(c) Follow up of patient for any future reclassification of the variant

It is very important for the clinician to understand that all VUSs do not need to be immediately interpreted in the absence of convincing evidence. In such situations the best practice would be to follow up the patient at specific intervals and do reanalysis of data **(Figure 2B)**. VUS reclassification is likely to happen as more cases with the condition get reported and more information is available about the functional significance of variants in a particular gene.

Exome sequencing has revolutionized the field of genetic diagnostics. However, it is important that clinicians observe precautions while interpreting a VUS, since a wrong interpretation is likely to cause more harm to the patient than an absence of diagnosis.

References

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Table 1 Examples of reverse phenotyping

Clinical features	Variant identified	Disease suspected	Reverse phenotyping
Clinical and radiological features consistent with Mucopolysaccharidosis	VUS in IDS (iduronate-2-sulphatase) gene	Hunter syndrome (Mucopolysaccharidosis Type II)	Iduronate 2-sulfatase (IDS) enzyme assay in white blood cells from peripheral blood, fibroblasts, or plasma (IDS enzyme level will be low with normal activity of at least one other sulfatase).
Clinical findings consistent with limb girdle muscular dystrophy (LGMD) and raised serum creatine kinase(CPK)	VUS in CAPN3 (calpain 3)gene	Calpainopathy (LGMDD4/ LGMDR1)	Muscle biopsy and immunoblot analysis for documenting reduction or absence of calpain 3 protein