How We Use Genetic Pathogenic Variants and Variants of Uncertain Significance (VUS) in Clinical Practice

Aaisha Al-Balushi[1], Maria Al-Hinai[1], Khalid Al-Thihli[2], Zandre Bruwer[2,4], Ghada A. Otaify[2], Katta M. Girisha[3], Almundher Al-Maawali[2,3]*

[1]National Genetic Center, Royal Hospital, Ministry of Health, Muscat, Oman

[2]Department of Genetics, Sultan Qaboos University Hospital, University Medical City, Muscat, Oman

[3]Department of Genetics, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman

[4]Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and University of Cape Town, Observatory, South Africa

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*Corresponding Author: almaawali@squ.edu.om

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Abstract

Accurate interpretation of genetic variants identified through sequencing technologies is essential for delivering precise diagnostics, informed therapeutic decisions, and effective genetic counseling. While pathogenic variants are directly actionable, variants of uncertain significance (VUS) present a clinical challenge and require comprehensive evaluation, including clinical correlation, database review, functional assays, and familial segregation analysis, to clarify their clinical relevance. In this context, two major medical genetics centers in Oman have collaboratively developed local guidelines aimed at uniform clinical practice and minimizing diagnostic inconsistencies due to subjective (clinician) bias. These recommendations emphasize the importance of clinician-laboratory collaboration, population-specific data, and adherence to the latest international variant classification frameworks. The guidelines aim to foster genomic literacy, ensure consistent clinical practice, and support cost-effective reflex testing and are particularly relevant for consanguineous populations.

Background and Purpose

The accurate interpretation of genomic variants identified through state-of-the-art sequencing technologies is crucial for delivering effective diagnostic, therapeutic, and counseling services. Pathogenic variants are considered clinically actionable for diagnosis and management. Variants of Uncertain Significance (VUS) require further clinical evaluation, review of genetics databases, functional studies, and family-based genetic testing to elucidate their clinical relevance. Although challenging, we strive to develop local guidelines to ensure that patients receive the most informed care, based on the current understanding of genetic and molecular data, and to minimize clinical decisions potentially influenced by clinical ascertainment bias. The authors from two large medical genetic centers in Oman reviewed the draft. They deliberated on various aspects mentioned in this document, and intend to use it at both centers to ensure uniform clinical practice. We believe sharing these recommendations is particularly relevant for other clinicians in the Middle East and other populations where medical genomic literacy is still in development. The recommendations also aspire to guide cost-effective utilization of resources for reflex genetic testing.

We recognize that the interpretation of a variant for clinical decision-making requires comprehensive knowledge of the patient's phenotype, the mode of inheritance of the disease gene/region, the mechanism of disease-causation (e.g., haploinsufficiency, dominant negative effect, or dosage sensitivity), protein structure and function, and the strength of the genotype-phenotype association. Collaboration between clinicians and laboratory scientists is essential for high-quality

variant classification. Clinicians must provide adequate, precise, and specific clinical information (using Human Phenotype Ontology terms) to enable the geneticists and clinical scientists to interpret genomic data accurately. In a consanguineous population, the locally defined population-specific allele frequency and segregation data from other affected families are particularly valuable for accurate variant interpretation.

These guidelines provide a systematic approach for the use of Pathogenic or Likely Pathogenic variants (hereafter referred to as Pathogenic variants) and Variants of Uncertain Significance (VUS) identified via genomic sequencing technologies. We made efforts to incorporate current best practices and resources, focusing on accurately interpreting genetic findings to guide clinical decision-making, diagnosis, and management strategies. The classification of variants must adhere to established clinical standards. The American College of Medical Genetics and Genomics and the Association of Molecular Pathologists (ACMG/AMP) and the Association for Clinical Genomic Science (ACGS) are two widely used systems.[1,2] Clinical Genome Resource (ClinGen) provides continuous updates on these guidelines.[3]

Clinical Use of Pathogenic Variants:

Pathogenic variants are those with sufficient evidence supporting their causal role in disease. These variants are associated with clinically recognized phenotypes and can be classified as disease-causing or likely disease-causing based on clinical and laboratory evidence, computational data, functional studies, and population frequency. Consult the flowchart illustrating the clinical application of pathogenic genetic variants in diagnosis, patient management, and genetic counseling (Supp 1).

Step 1: Clinical Correlation

- Conduct a comprehensive clinical evaluation to ensure the phenotype aligns with the disease associated with the identified variant.

- If phenotypic discordance is observed, consider alternative diagnoses or re-evaluate the clinical presentation or the variant classification. Additional diagnostic tests may be required to confirm the diagnosis or rule out co-existing conditions.

- Caution is advised for genetically heterogeneous and nonspecific phenotypes, such as intellectual disability, epilepsy, and developmental delay, as the clinical specificity is lower. Clinicians should also be aware of age-dependent penetrance for some of the anticipated clinical features.

Step 2: Consider Inheritance Patterns

A. Autosomal Dominant Inheritance (Heterozygous Pathogenic Variant)

1. Parental Testing

- If the condition is known to occur mostly *de novo* without significant variable expressivity or penetrance, parental testing is not required for a known pathogenic variant that has been documented to have occurred *de novo* in other families (as reported in the literature). Recurrence risk can be estimated based on epidemiological data.

- For newly reported conditions or those with significant variability in expression or penetrance, test both parents. If both are negative for the pathogenic variant, this suggests a *de novo* mutation in the proband, and further family testing is unnecessary unless family history shows recurrence of the same condition, suggestive of germline mosaicism.

- If either parent carries the variant classified as pathogenic, determine whether the individual carrying this variant is symptomatic or exhibits milder manifestations. If not, provide a well-documented evidence-based reason for the absence of clinical

manifestations (e.g., reduced penetrance, late age of onset, variable expressivity, known modifier genes, incorrect variant classification, or lab errors). If the absence of clinical features cannot be explained, consider requesting the laboratory to reclassify the variant or downgrade its classification.

2. *Affected Family Member**:** Offer genetic testing to confirm the diagnosis and tailor surveillance recommendations.*

3. Unaffected Family Member: Provide counseling on disease onset, penetrance, and implications of predictive or pre-symptomatic testing. Offer testing to assist with family planning and monitoring for disorders that have an impact on pre-symptomatic interventions or serious clinical sequelae once manifested, ensuring that the process adheres to ethical guidelines, especially when considering testing minors.

B. Autosomal Recessive Inheritance (Homozygous or Compound Heterozygous Pathogenic Variants)

1. *Parental Carrier Status**:***

- o **Obligate Carriers**: Parents of an individual affected by an autosomal recessive condition are considered obligate carriers of the disease-causing homozygous genetic variant. Additional testing for the parents is not required and is not indicated. However, testing is only indicated if the parents have confirmed they will consider primary prevention options, namely Prenatal Genetic Diagnosis (PND) and Preimplantation Genetic Testing (PGT).

- o **For presumed compound heterozygosity**, testing parents would be indicated to determine whether the two identified variants are inherited in *cis* (both from the same parent) or *trans* (one from each parent). Only variants in *trans* are typically considered pathogenic and disease-causing.

2. *Testing of affected siblings **or** family members with the same phenotype is not indicated and does not require additional genetic testing for confirmation of the diagnosis once the pathogenic variant is identified in the proband. Testing is indicated if a major therapeutic intervention is planned, for example, organ transplantation. Be aware of morbidities and clinical manifestations that are unexplained by phenotypic variations, as recognized with confirmed diagnoses and multiple diagnoses in the same individual.*

3. *Testing of Apparently Healthy Siblings**:***

- o Healthy siblings can be tested for carrier status only after the age of 18 years, to inform premarital screening and assist with family planning.

- o Testing asymptomatic children under the age of 18 years is indicated for disorders where pre-symptomatic intervention or screening is clinically indicated, and there is no alternative highly sensitive diagnostic method available, or the latter has yielded ambiguous results.

4. *Testing of Extended Family**:***

- o Carrier testing should also be considered for other family members for premarital screening or to provide appropriate genetic counseling and surveillance.

C. X-linked inheritance (Hemizygous or Heterozygous Pathogenic Variants)

1. *Parental Carrier Status*

- o For X-linked dominant conditions without variable expression or incomplete penetrance, testing is not indicated. Usually, these conditions are lethal in males and severe in females, albeit for some exceptions.

- o For X-linked recessive conditions or cases with variable expression, maternal testing is recommended to estimate recurrence risks or inform management of carriers.

2. *Testing of Affected Siblings: Further testing of siblings with the same phenotype is unnecessary after the proband's variant is identified. Be careful to confirm that the phenotype is the same as the proband's.*

3. *Testing of Apparently Healthy Siblings or Extended Family: Carrier testing should be offered (gender-specific) after the age of 18 years to assist with premarital screening and family planning.*

4. *Testing asymptomatic relatives under the age of 18 years is indicated for disorders where pre-symptomatic intervention or screening is clinically indicated, and there is no alternative highly sensitive diagnostic method available, or the latter has yielded ambiguous results.*

Clinical Use of Variants of Uncertain Significance (VUS):

A VUS is a genetic variant for which evidence is insufficient to classify it as benign or pathogenic. Reclassifying a VUS is a complex, time-consuming, and expensive process that often requires extensive literature review, functional studies, family segregation analysis, and/or data sharing across laboratories and databases. This process is further complicated by the need for longitudinal data collection and reinterpretation over time as new evidences emerge. Importantly, experience and accumulated data have shown that the majority of VUSs are ultimately reclassified as benign or likely benign.[4-6] As a result, laboratories and clinicians must weigh the investment of time and resources against the relatively low clinical yield of actionable findings from VUS reclassification. The following steps aim to gather further evidence to better understand their clinical relevance.

Step 1: Initial Evaluation of VUS

1. *Reassessment of VUS*

- o Review new evidence from updated genomic databases (e.g., ClinVar, gnomad, Decipher) and published literature.

- o If reclassified as benign, likely benign, or VUS with weak evidence, further testing is not required.

2. *Comprehensive Clinical Assessment*

- o Perform detailed phenotype-specific investigations (e.g., neuroimaging, muscle biopsy, biochemical assays).

- o Utilize these findings to evaluate whether the VUS contributes to the disease phenotype.

3. *Segregation Analysis*

- o Conduct segregation studies to determine whether the VUS co-segregates with the disease phenotype in affected family members initially and then in other family members, like healthy siblings or unaffected parents.

- Remember to take into account the inheritance pattern, variable expressivity, and penetrance. These are some factors that will complicate the reclassification if not clarified from the start.

4. *Therapeutic Trials*

- Consider trying clinically indicated therapeutic interventions targeting the suspected mechanism imposed by the VUS if assumed to be pathogenic. Monitor patient response to treatment for insights into variant pathogenicity. This new information can then be used in reclassification.

Step 2: Variant Interpretation and Classification

After collecting sufficient clinical, genetic, and functional data, refer the case to a clinical molecular genetics laboratory for reclassification. The accepted variants classification systems, like ACMG/AMP or ACGS, should be used. This process should consider:

- New functional studies
- Updated genomic databases
- Family segregation studies (including information from other similarly affected local populations and tribes) and
- Clinical outcomes from therapeutic interventions

If the above-mentioned steps fail to reclassify the VUS as pathogenic, and it remains a VUS, we recommend that it not be used for interventional therapeutic management or preventive measures, such as PGT and PND.

Management of Specific Scenarios Involving VUS:

1. *Recessive Disorders with One Pathogenic Variant and One VUS*

- In autosomal recessive disorders, where one allele harbors a pathogenic variant and the second allele contains a VUS, perform a full assessment as indicated above and follow the steps mentioned (section: Initial Evaluation of VUS).

2. *VUS Unrelated to the Phenotype*

- If the VUS does not correlate with the patient's clinical phenotype and no further evidence emerges, no further action is required. The variant should remain classified as VUS until new data is available.

- Document the lack of clinical significance in the patient's medical records and continue to monitor for gene-disease association updates in the literature and databases.

3. *VUS linked to conditions requiring surveillance, for example, cancer risk or other surveillance programs (e.g., cardiac conditions): Follow the steps above to reclassify the variant if possible (section: Initial Evaluation of VUS). However, remember to take into account the inheritance pattern, variable expressivity, and penetrance. These are factors that will complicate the reclassification. In this case, clinical discretion should be applied regarding segregation analysis, as well as in accordance with clinical guidelines for surveillance. The test showing a VUS that cannot be reclassified should be considered a negative test.*

4. *VUS in genetic counseling practice: Regarding carrier screening, premarital testing, or primary prevention strategies, testing for VUS in these contexts is not indicated and should be actively discouraged by the genetic counseling teams. The primary medical physician overseeing the case may pursue efforts toward reclassification when appropriate. While genetic counseling can still address*

VUS findings, discussions should emphasize the uncertainty of their clinical significance. Risk assessment and recurrence risk counseling may consider the possibility that a VUS is causative, but such interpretations must be framed with caution. Premarital testing should not include VUSs; however, counseling may explore other options to reduce the risk of autosomal recessive conditions of unknown etiology. Use of VUSs in PGT or PND is not appropriate and should be avoided.

In conclusion, accurate interpretation of genetic variants, especially VUS, remains a clinical challenge, prompting collaborations to develop population-specific guidelines that promote standardized, evidence-based variant classification and foster clinician-laboratory collaboration. We hope this guideline to be useful to enhance molecular diagnostic consistency and care in consanguineous populations and beyond.

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