Supplemental Digital Content-1

Variant of uncertain Significance (VUS) subcategorization

The overall variant of uncertain significance (VUS) designation denotes a variant with a probability of pathogenicity of 10 - <90%. Because variants are classified using semiquantitative criteria, there is a nearly continuous range of likelihoods of pathogenicity within that range. However, in a *diagnostic* testing scenario, where the prior probability of disease is high, variants at the high end of that range can be useful for the clinician because for some, a small increment of additional data for pathogenicity (e.g., another affected family member) could make the variant diagnostic.

To facilitate this, many clinical testing laboratories subcategorize VUS for use in diagnostic testing. The American College of Medical Genetics and Genomics, ClinGen, the College of American Pathology and the Association for Molecular Pathology have released a draft of their upcoming recommendations for variant classification that formalizes this approach. They have proposed adoption of the ClinGen recommendation of the Bayesian points approach[1], which is in turn an adaptation of the Bayesian variant classification system[2]. In that system, a variant that garners from 0 to +5 evidence points is classified as a VUS. This allows ready subdivision, such that +4 to +5 points would be designated VUS-Hi, +2 to +3 points would be designated VUS-Mid, and 0 to +1 points would be designated VUS-Lo. These points designations correspond to probabilities of pathogenicity of 68-84% (VUS-Hi), 33-50% VUS-Mid, and 10-19% VUS-Lo.

Critically, these probabilities are the likelihood of pathogenicity of the variant. They are not the probability that an individual harboring that variant has the associated disease. This difference can be enormous. It is in one sense, a consequence of biased ascertainment. When a group of patients have numerous symptoms of a disorder and a genetic test identifies a variant in them, their likely disease status biases the pool of variants that is found among that group towards pathogenic variants. When a group of patients have instead have no symptoms of a disorder and a genetic test identifies a variant in them, their likely disease status biases the pool of variants that is found among that group towards benign variants. Unfortunately, this bias cannot be quantified for malignant hyperthermia susceptibility – the current estimates of the prevalence (frequency of the disease in the population) and the penetrance (likelihood of a malignant hyperthermia (MH) reaction for a person with MH susceptibility (MHS) when exposed to a triggering agent) of the trait are too unreliable.

However, the phenomenon can be illustrated by calculations for another disorder, hereditary breast and ovarian cancer. The detection of a likely pathogenic variant in breast cancer gene-1 (*BRCA1)* (90% probability of pathogenicity) in a woman with ovarian cancer at 35 years of age, gives a diagnostic likelihood of hereditary breast and ovarian cancer of >99.9%. The detection of a likely pathogenic variant in a healthy 35-year-old woman with multiple relatives without breast and ovarian cancer gives a diagnostic likelihood of less than 20%. The detection of a VUS-Hi variant would yield a diagnostic probability in the range of 2%. A VUS-Mid would be substantially lower than that.

As well, there is strong evidence that the detection of likely pathogenic and pathogenic variants among apparently healthy people is associated with lower penetrance, compared to individuals diagnosed from families that are affected by MHS. Indeed, some studies of MHS genomics in populations show frequencies of these variants in as many as 1 in 800 individuals[3]. Given that MH reactions occur in 1 in 30,000 to 1 in 100,000 exposures, this would suggest a penetrance of 1 to 3%. Were MHS to have the same diagnostic yield as breast and ovarian cancer and a penetrance of 3%, this would suggest a likelihood of a reaction in a person harboring a VUS-Hi variant would be less than 1 per 5,000. It would be substantially lower for a person harboring a VUS-Mid variant.

We have recommended extending precautions for MHS to individuals who harbor VUS-Hi variants, even if they have no personal or family history of MH or MHS, even though these numbers suggest the risks are low. Given that the risks of a VUS-Mid variant would be yet lower, we suggest that these are prudent and defensible recommendations.

REFERENCES

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