CLINICAL SUMMARY

Spectrum of splicing variants in disease genes and the ability of RNA analysis to reduce uncertainty in clinical interpretation

Large retrospective study examines the role of RNA analysis for variant interpretation in germline genetic testing

Invitae takeaway

- Assessing the clinical consequences of splicing variants can be challenging. Of the patients tested at Invitae across all clinical areas, 5.4% harbored a splicing VUS.
- Based on Invitae's variant interpretation framework, Sherloc, 1.7% of the entire cohort had a splicing VUS that could be made definitive by RNA analysis.
- The expanding use of RNA analysis in hereditary disease testing will improve the clinical sensitivity of genetic testing.

Overview

Splice sites are specific DNA sequences found in every gene. When variants occur within splice sites of disease genes, it is challenging to know which ones will adversely affect a patient's health. Many will have no effect on the gene function; others may affect gene function but not lead to any medical outcomes; and yet others will have significant impact on health. RNA analysis of splicing variants has been identified as a method to distinguish harmful splicing variants from benign ones. An expansive study conducted by Invitae, and published in *The American Journal of Human Genetics*, sought to examine the impact of RNA analysis on resolving variant(s) of uncertain significance (VUS) in splicing regions.

Study design

This retrospective study had three arms. The first arm investigated the number of splicing VUS that could be definitively classified with RNA analysis in a clinical cohort of 689,321 patients previously tested for a variety of inherited conditions across all clinical areas. The second arm examined the number of splicing VUS in ClinVar, a public archive of genetic variants and observed phenotypes. The third arm explored the diversity of splicing variants found in healthy individuals in the Genome Aggregation Database (gnomAD), a public archive of exome and genome sequencing data.

Relevant findings

In the Invitae clinical cohort, 5.4% of patients had splicing variants that were classified as VUS and therefore had an uncertain impact on health. When incorporating evidence gained from RNA analysis into Invitae's variant interpretation framework (Sherloc), 32% of patients with a splicing VUS were projected to receive a definitive result. This was 1.7% of the cohort. Most of the reclassifications would result in a "likely benign" interpretation, in part due to the fact that most splicing VUS were in intronic regions that are often tolerant to change. RNA analysis could potentially provide a likely pathogenic result (and one that is clinically actionable) for 2.5% of patients with a splicing VUS, or 0.1% of all patients tested.

The ClinVar database was then used to examine how the Invitae internal findings compared with those from other labs. In ClinVar (excluding Invitae submissions), splicing VUS reported by 95 laboratories were 4.8% of all reported variants, suggesting that the genetic testing community as a whole has a need for splicing VUS resolution.

Based on data from gnomAD, splicing variants make up 9.4% of all variants in healthy individuals, indicating that some splicing variants are compatible with normal gene function. Thus, the clinical significance of splicing variants must be interpreted cautiously, as they are not always pathogenic.

Publication

1. Truty *et al.* Spectrum of splicing variants in disease genes and the ability of RNA analysis to reduce uncertainty in clinical interpretation. *The American Journal of Human Genetics. In press.* Published online March 19, 2021. https://doi.org/10.1016/j.ajhg.2021.03.006
2. Nykamp K, *et al.* Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria *Genetics in Medicine.* 2017 Oct;19(10):1105-1117. doi: 10.1038/gim.2017.37. Epub 2017 May 11.