Silent Variants Speak: Variants of Unknown Significance Alter RNA Splicing in Inherited Cardiomyopathy

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Background: Sequence variants that create or eliminate splice sites are often clinically classified as variants of unknown significance (VUS) due to imperfect understanding of RNA splice signals and cumbersome functional assays. In autosomal dominant disorders caused by haploinsufficiency, variants that alter normal splicing of one allele are pathogenic. We sought to improve diagnostic sensitivity of clinical genetic testing by identifying unknown splice-altering variants in two established cardiomyopathy disease genes.

Methods: We developed enhanced computational tools to prioritize potential splice-altering VUS, and used minigene assays to functionally confirm splice-altering sequence variants. We studied all rare variants reported in large reference and patient databases for lamin A/C (LMNA) and myosin binding protein C (MYBPC3), as haploinsufficiency of these genes causes dilated cardiomyopathy with associated conduction defects, and hypertrophic cardiomyopathy, respectively.

Results: 1162 rare reference variants and 1228 rare clinical variants across both genes were available for study. The proportions of assay-validated splice variants differed between the reference and clinical databases (0.4% vs. 3.9%, p=1.4e-09). We identify 13 LMNA and 35 MYBPC3 rare variants in clinical databases that alter normal RNA splicing, which correspond to a 50% increase in damaging splice variants in affected patients. Over half of these variants are annotated as VUS by clinical diagnostic laboratories. Further analyses of one variant, a synonymous VUS in LMNA, showed familial segregation with cardiomyopathy and altered LMNA RNA splicing in both lymphocytes and affected heart tissues.

Conclusion: Bioinformatic prioritization of VUS combined with cell-based functional analyses improves clinical detection of human pathogenic variants in cardiomyopathy genes. This strategy should be broadly relevant to other autosomal dominant human disorders that are caused by haploinsufficiency.