Impact Analysis of PVS1 Criteria on Canonical Splice Variant Classifications

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Background

- Most canonical splice site variants, located at the +/- 1, 2 positions, are considered disease-causing based on the nature of the mutation and are predicted to result in skipping of the adjacent exon.

- However, recently it has become clear that additional factors surrounding these variants should be considered for classification. This includes the addition of ACMG/AMP pathogenic criteria (PVS1) for predicted loss of function[1].

- These guidelines were incorporated into our classification process and applied to variants identified as part of the Foresight™ Carrier Screen. Here, we evaluate the impact of this update on classification of canonical splice variants.

1. Tayoun ANA, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, Harrison, SM, ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI). Recommendations for interpreting the loss of function PSV1 ACMG/AMP variant criterion. 2018. *Hum Mutat*. 39(11):1517-1524.

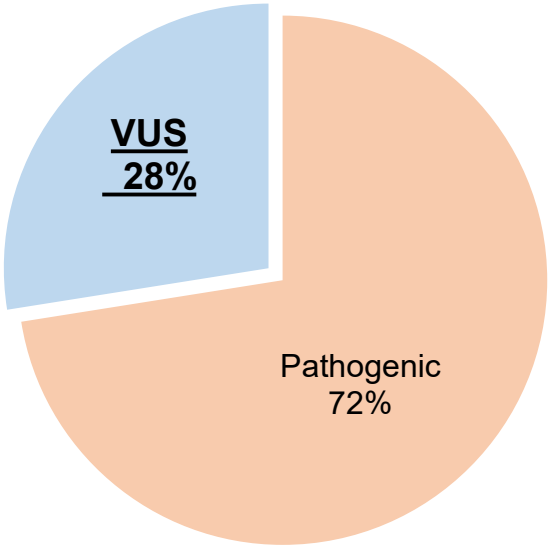
Methods

- The analysis herein included 1,215 unique canonical splice variants observed in 176 genes curated between November 2018 and May 2020.

- We sorted the data by evaluating exon frame, whether the region is critical for protein function, the percentage of the protein loss, the presence of a cryptic site and evidence for reconstitution of the splice site.

- Additional evidence was also collected including the presence of cases and *in-silico* splice tools for Neural Network Splice Site Prediction (NNS), Splicing Site Finder (SSF) and MaxEntScan (MES).

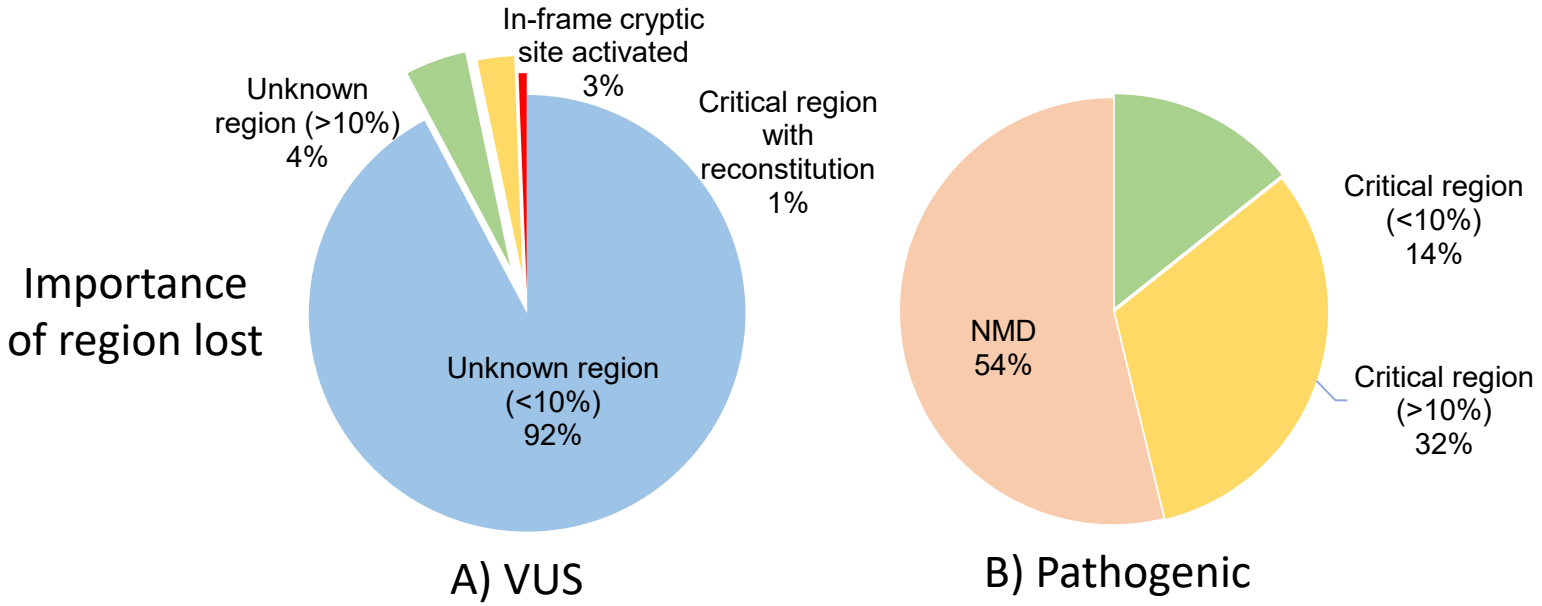
Results



Classification of variants

A) 334 variants (27.5%) were classified as a variant of unknown significance (VUS), and 881 (72.5%) were classified as pathogenic. A large portion of pathogenic variants (53.8%) were predicted to be subject to nonsense mediated decay (NMD).

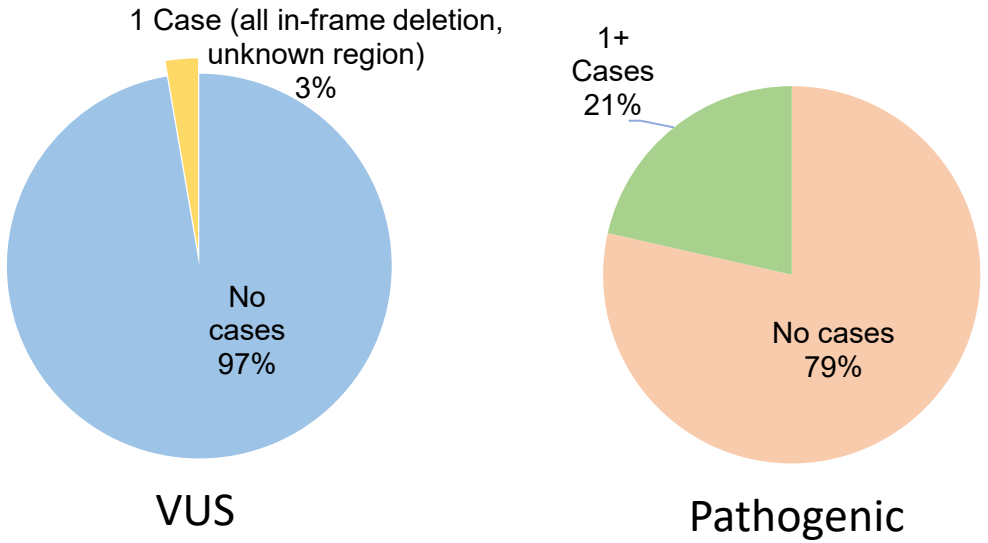
Results



A) Within the VUS group, 96.7% were found in a region with unknown function. Two variants were found in a critical region but were classified as VUS due to predicted reconstitution of the canonical splice site.

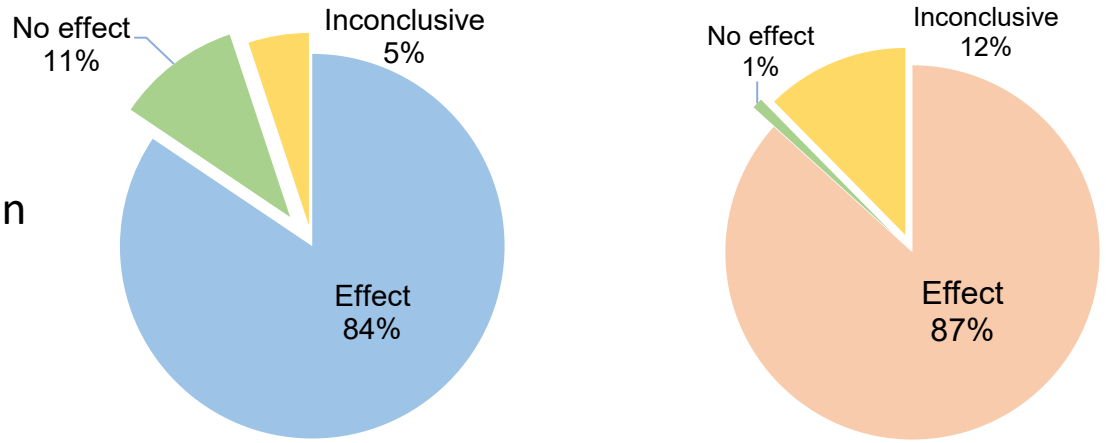
B) The pathogenic variants not predicted to undergo NMD had evidence that the lost region was critical.

A) Presence of cases



VUS Pathogenic

B) *In silico* prediction effect on splicing



VUS Pathogenic

- A) Within the VUS group 97% had no cases vs 79% within the pathogenic group had no cases.
- B) Within the VUS group 11% were found to have no predicted effect on splicing vs 1% of the pathogenic group had no predicted effect.

	Critical Region		Unknown Region		In-frame cryptic site activated	Total
% protein removed	**>10%**	**<10%**	**>10%**	**<10%**		
Pathogenic	281(69%)	126(31%)	0(0%)	0(0%)	0(0%)	407*
VUS	1(0.3%)	1(0.3%)	15(4.5%)	308(92.2%)	9(2.7%)	334
Total	282	127	15	308	9	741
*** 474 pathogenic variants were subject to NMD and not included in the table above**						

Table 1: Summary of canonical splice variants classified with PVS1 criteria

- Summary of the characteristics of variants classified as pathogenic or VUS. Note in this table pathogenic variants subject to NMD are excluded.

Conclusions

- In summary (Table 1), most canonical splice variants classified as VUS were predicted to have no impact on reading frame, were in a region of unknown function and remove <10% of the protein (92.2%).

- Within the pathogenic group, over 50% were predicted to be subject to NMD (54%). Of the remaining pathogenic variants not predicted to be subject to NMD, all removed a critical region and most removed >10% of the protein (32% with NMD variants included in count, 69% when NMD variants are removed).

- This analysis has provided valuable insight into the different aspects of canonical splice site variants that contribute to classification and demonstrate our commitment to providing the most accurate and up-to-date classification for our patients.