CLARIFICATION OF VARIANT REPORTING FOR HOMOLOGOUS GENES RESOLVED THROUGH SYSTEMATIC LITERATURE REVIEW – ACMG SF GENES *CALM1*, *CALM2*, AND *CALM3*

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Introduction

Challenges associated with variant interpretation are compounded when disease-causing genes have homologous counterparts. This phenomenon makes variant calling, evidence curation, and diagnostic interpretation especially error-prone. Homologous-annotation, the application of pathogenic criteria in variants across homologous genes with related/identical protein-function, is necessary to minimize missed diagnoses.

Here we report on the findings of semi-automated curation of the published dataset for the American College of Medical Genetics and Genomics (ACMG) guidelines secondary finding (SF) genes *CALM1, CALM2, and CALM3 (CALM)*.These recent additions to the ACMG SF list, associated with severe calmodulinopathies, encode an identical calmodulin protein and only differ in their promoter and untranslated regions. Each *CALM* gene resides on a distinct chromosome: *CALM1* - chr14q31, *CALM2* - chr2p21, and *CALM3* - chr19q13 (see Figure 1). Homologous-annotation (HA) was used in the curation of the *CALM* dataset and serves as an important example of the benefit of reconciling variant annotations across homologous genes.

Figure 1: Clustal Omega Multiple sequence alignment of Calmodulin translated protein products

Methods

Published journal articles with at least one variant in *CALM*, as identified by the Mastermind Genomic Intelligence Platform, were evaluated with additional assessment of clinically-encountered variants in ClinVar. Variant nomenclature was normalized according to the Uniprot associated RefSeq canonical transcripts, *CALM1* (NM_006888.6), *CALM2* (NM_001743.6), and *CALM3* (NM_005184.4). Articles were reviewed to identify any clinical or functional evidence according to the ACMG guidelines.

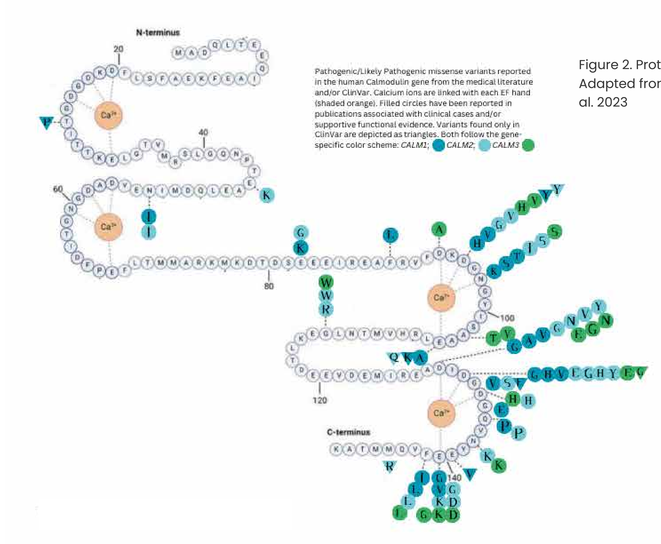

Figure 2. Protein Structure Adapted from Hussey et al. 2023

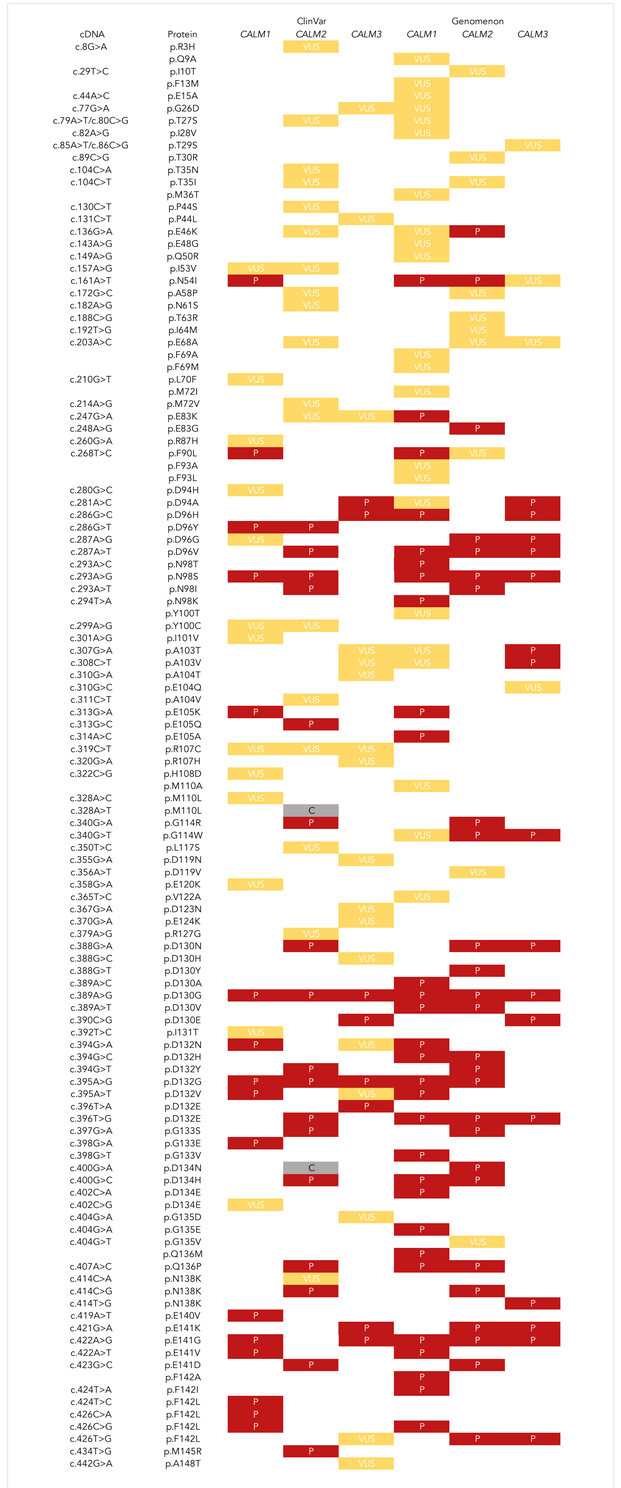

Figure 3: Heat map showing the distribution of genetic variants across *CALM* genes in ClinVar and found in the literature designated as "Genomenon". The presence and variant classification calls were represented as white (absent), yellow (VUS), gray (Conflict call), and red (P/LP) prior to the application of HA

Results

In total, 114 unique variants were identified in *CALM* genes from our meta-analysis and curation of the literature and associated databases. Of these, 71 were designated pathogenic (P/LP) in instances of an individually cited *CALM* gene. Inclusion of unique ClinVar entries of P/LP variants brings the variant total to 100. publication.

Among the 66 unique P/LP variants; 29 *CALM1*, 26 *CALM2*, and 16 *CALM3* variants were identified in the literature. As *CALM* genes encode the same protein with similar cardiac expression one can imply that a P/LP variant in one gene would be causative to the other CALM genes. The application of HA across published *CALM* genes increased total P/LP variant count by an average of 197.9% (61 total variants across all three genes; *CALM1* =127.6%; *CALM2* = 153.8%; *CALM3* = 312.5%), see Figure 4.

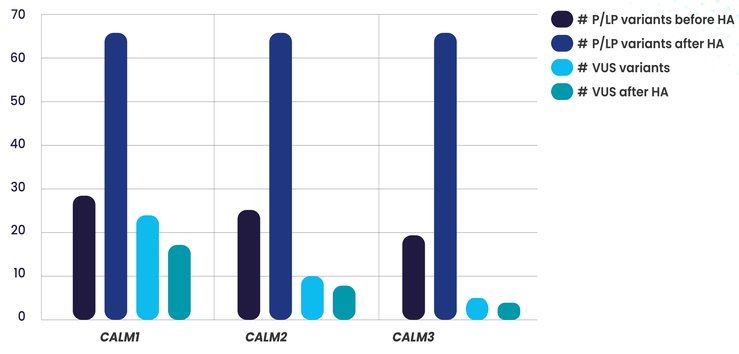

Figure 4: Variant Calls by method

Conclusions

We have completed a systematic literature analysis, database reconciliation, and HA of the *CALM* genes which underlie the difficulties in classifying *CALM* variants from individual probands, whose inheritance is typically de novo, especially in a priori risk settings. The transfer of annotations through HA would increase diagnostic rates, decrease VUSs, reduce conflict calls, and could be implemented in clinical databases. Leveraging existing frameworks, our data delivers important insights.

- ClinGen's Rasopathy Variant Curation Expert Panel (VCEP) recommends PS1, PM5, and PM1 criteria to apply to specific Rasopathy genes for analogous residue positions/regions in highly analogous groupings[2]. Variant annotation across homologous proteins has been studied and termed "Paralogue Annotation (PA)" for those homologous protein domains that are not fully homologous protein sequences[4-5].

- ClinGen has also conducted gene association assessment of the CALM genes. The expert group applied a similar approach for combining evidence for strength of association across all three genes[3].

Evidence suggests that this approach of HA, or PA for those without complete sequence homology but regional similarities, should be considered at both the GDR assessment and variant curation level.

References

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