Using Quantitative Variant Classification to Tackle the VUS Problem in Genetic Hearing Loss

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Introduction

- Genetic testing is essential for the care of deaf and hard-of-hearing individuals. The American College of Medical Genetics and Genomics (ACMG) recommends a multigene panel for patients with non-syndromic hearing loss (HL), which frequently identifies variants of uncertain significance (VUS).
- The ClinGen Hearing Loss Variant Curation Expert Panel (HL-VCEP) adapted ACMG guidelines for hearing loss genes. However, limitations remain, including the high prevalence of VUS and the ambiguity in classifying variants with both pathogenic and benign criteria.
- To address these issues, the ACMG working group is developing version 4 of the classification criteria (ACMG v4), which aims to establish a quantitative scoring system and refine VUS categorization into low, mid, and high subcategories.

This study aims to assess the utility of the forthcoming ACMG v4 guidelines in resolving the challenge of variants of uncertain significance as compared to the current ACMG v3 based on the analysis of 300 missense variants from individuals with hearing loss tested on a multigene panel.

Results

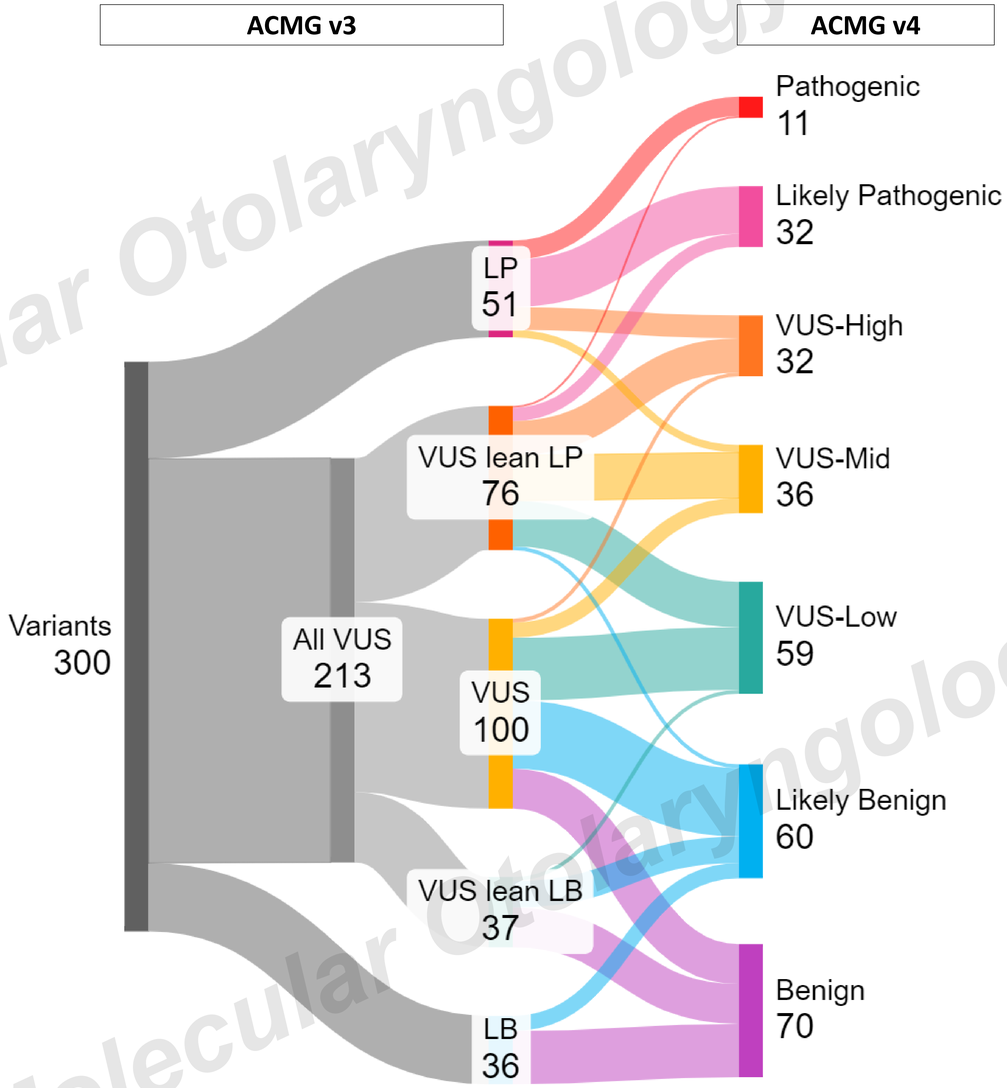

Figure 2. Reclassification of 300 missense variants from 99 hearing loss-associated genes using ACMG v4 criteria. Under ACMG v4, ~4% of VUSs were upgraded to P/LP while ~57% were downgraded to B/LB. ~10% of VUSs leaning LP were reclassified as LP while ~34% were downgraded to VUS-Low or LB. Of the Likely Pathogenic variants under v3, ~20% were upgraded to Pathogenic and ~31% were downgraded to a VUS.

Methodology

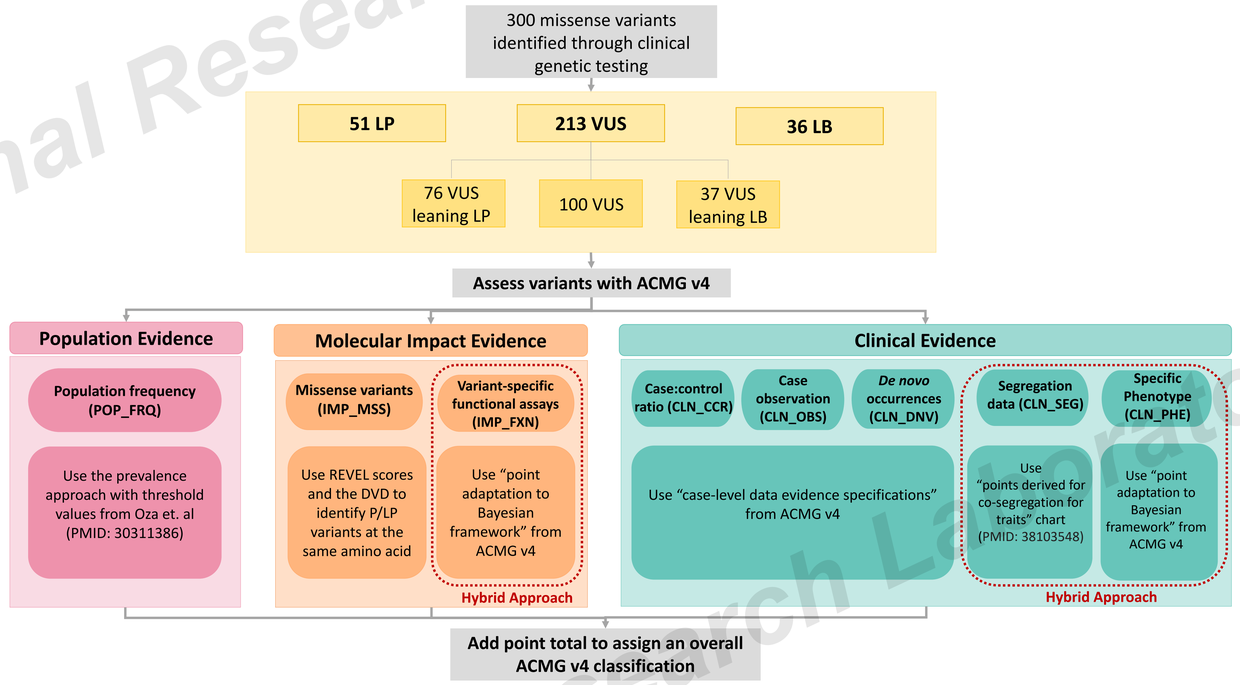

Figure 1. Reclassification workflow of 300 missense variants using ACMG v4 criteria. A hybrid approach to scoring was applied for three types of evidence due to the absence of defined guidelines in the ACMG v4 criteria. DVD: Deafness Variation Database (https://deafnessvariationdatabase.org/)

VUS Reclassification Drivers

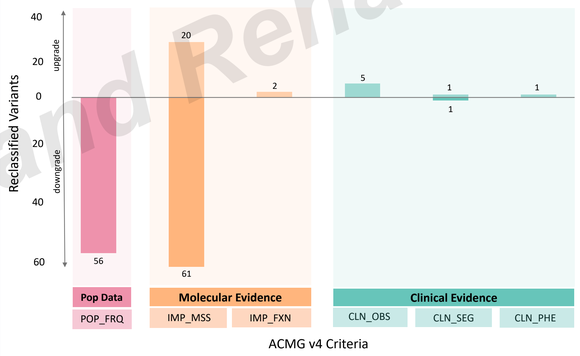

Figure 3. Drivers of VUS Reclassification. Of the 213 missense VUSs assessed using ACMG v4, 102 were downgraded or upgraded. The ACMG v4 criteria most frequently applied in reclassifying these variants were POP_FRQ (population evidence) and IMP_MSS (prediction data using REVEL scores).

Example Classification

KCNQ4: NM_004700.4: c.778G>A, p.(Glu260Lys)

Data		ACMG v3	ACMG v4
Population Frequency	• MAF: not reported in gnomAD	PM2_Sup	POP_FRQ_0
Molecular Impact	• CADD: 32.0, REVEL: 0.96 • No P/LP variants at the same AA • *In vitro* functional studies show that this variant results in reduced channel function (PMID: 31995783)	PP3, PS3_Sup	IMP_MSS_+4, IMP_FXN_+1
Clinical Evidence	• Variant has been reported in 5-year-old proband with mild-to-moderate sensorineural hearing loss (PMID: 18941426) • Variant identified in a MORL proband with congenital mild SNHL	PS4_Sup	CLN_OBS_+1
Classification		VUS lean LP	LP (6 points)

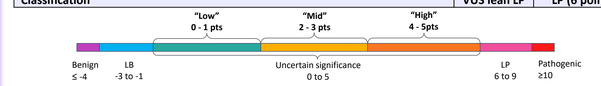

Table 1. Classification data of 1 proband upgraded to a positive genetic diagnosis with ACMG v4 criteria

Conclusions

- Variant classification using ACMG v4 criteria *significantly reduces* the number of VUSs.
  - **57%** of VUSs were downgraded to Likely Benign or Benign.
- ACMG v4 subcategorization of VUS improves the identification of clinically relevant genetic variants, thereby enhancing patient care.
  - **~2% (5)** of VUSs in 3 genes were upgraded to Likely Pathogenic, resulting in a genetic diagnosis for 5 probands.
- The most significant factor driving VUS variant reclassification using ACMG v4 was molecular impact evidence based on prediction data (REVEL scores), followed by population data.
- **Further refinement of the ACMG v4 guidelines, especially in weighting molecular impact and clinical evidence, is essential for improving variant classification accuracy and genetic diagnoses for hearing loss.**

References and Acknowledgements

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- https://clinicalgenome.org/affiliation/50007/

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