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BRIEF COMMUNICATION

Disease-specific ACMG/AMP guidelines improve sequence variant interpretation for hearing loss

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PURPOSE: The ClinGen Variant Curation Expert Panels (VCEPs) provide disease-specific rules for accurate variant interpretation. Using the hearing loss-specific American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines, the Hearing Loss VCEP (HL VCEP) illustrates the utility of expert specifications in variant interpretation.

METHODS: A total of 157 variants across nine HL genes, previously submitted to ClinVar, were curated by the HL VCEP. The curation process involved collecting published and unpublished data for each variant by biocurators, followed by bimonthly meetings of an expert curation subgroup that reviewed all evidence and applied the HL-specific ACMG/AMP guidelines to reach a final classification.

RESULTS: Before expert curation, 75% (117/157) of variants had single or multiple variants of uncertain significance (VUS) submissions (17/157) or had conflicting interpretations in ClinVar (100/157). After applying the HL-specific ACMG/AMP guidelines, 24% (4/17) of VUS and 69% (69/100) of discordant variants were resolved into benign (B), likely benign (LB), likely pathogenic (LP), or pathogenic (P). Overall, 70% (109/157) variants had unambiguous classifications (B, LB, LP, P). We quantify the contribution of the HL-specified ACMG/AMP codes to variant classification.

CONCLUSION: Expert specification and application of the HL-specific ACMG/AMP guidelines effectively resolved discordant interpretations in ClinVar. This study highlights the utility of ClinGen VCEPs in supporting more consistent clinical variant interpretation.

INTRODUCTION

Hearing loss (HL) is the most common congenital sensory condition, with approximately 50% of affected individuals having an identifiable genetic etiology [1]. HL is a heterogeneous condition with more than 100 genes underlying nonsyndromic HL, and over 400 genes implicated in syndromic forms of deafness [2, 3]. A clinical genetics evaluation is recommended as part of a standard of care diagnostic work-up, and results from genetic testing can often inform clinical management, especially if a genetic syndrome is identified before the onset of additional clinical manifestations [1, 4]. As an expert panel of the Clinical Genome Resource (ClinGen), a National Institute of Health (NIH)–funded resource focused on defining the clinical validity of gene and variant contributions to disease, the Hearing Loss Clinical Domain Working Group (HL-CDWG) (https://www.clinicalgenome. org/working-groups/clinical-domain/hearing-loss/), has worked to evaluate gene–disease relationships and standardize variant interpretation in hereditary HL [5–7].

Interpretation of variants' clinical significance is a rigorous process that involves collating and analyzing available literature and evidence, followed by a formal classification based on this evidence. The ClinVar database (https://www.ncbi.nlm.nih.gov/ clinvar/) archives and aggregates variant interpretations from various submitters and indicates whether the submitted interpretations are concordant or discordant. Data sharing through ClinVar provides an invaluable opportunity to identify classification differences and to collaborate with submitters to resolve these discrepancies. A pilot study conducted by four clinical laboratories showed that 87.2% (211/242) of discordant variants were resolved when the variant was reassessed with current criteria and/or through internal data sharing [8]. The resolution of conflicting interpretations helps provide crucial diagnostic information to clinicians and patients.

Here we demonstrate the successful implementation of the HL-specific ACMG/AMP guidelines [5] by the ClinGen HL Variant

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Curation Expert Panel (HL VCEP) to resolve discrepancies in variant interpretations.

MATERIALS AND METHODS

HL-CDWG organizational structure

The HL-CDWG has worked to create a standardized, thorough set of genes and variants that are associated with syndromic and nonsyndromic HL [5, 9]. The members include otolaryngologists, clinical geneticists, molecular geneticists, ClinGen biocurators, clinical researchers, and genetic counselors from 11 different countries including China, France, Israel, Japan, Netherlands, Singapore, South Korea, Spain, Tunisia, the United Arab Emirates, and the United States, representing 26 institutions. In order to accomplish this goal, the HL-CDWG formed two other groups defined by ClinGen as a Gene Curation Expert Panel (GCEP) and a Variant Curation Expert Panel (VCEP).

HL VCEP rule specification and curation process

A smaller task team of the HL VCEP worked to provide expert guidance for standardized variant interpretation and adapted the ACMG/AMP guidelines for the interpretation of sequence variants in nine HL genes, specifically *USH2A*, *SLC26A4*, *GJB2*, *MYO7A*, *CDH23*, *TECTA*, *COCH*, *KCNQ4*, and *MYO6* [5]. Those genes represent the major causes of hereditary hearing loss encompassing dominant and recessive inheritance models, different disease mechanisms, penetrance, and expressivities.

The HL VCEP group then continued to curate variants in these nine genes according to the new HL-specific standards with an emphasis on those that have conflicting interpretations in ClinVar (Fig. 1). The HL VCEP utilizes monthly meetings in addition to email correspondence to present, review, and reach consensus on the classification of these variants. Each variant was assigned to a single trained ClinGen biocurator who utilized various literature search engines and databases including PubMed, the Deafness Variation Database (DVD) [10], Human Gene Mutation Database (HGMD) [11], Google Scholar, Leiden Open Variation Database (LOVD) [12], and LitVar [13] to find publications associated with these variants. HL VCEP members also contributed case observations and phenotypic information from their laboratories or clinics. Additionally, ClinVar submitters were contacted via email to provide internal genotype and phenotype data for their submissions so that biocurators could aggregate all the relevant data. Curators utilized ClinGen's Variant Curation Interface (https://curation.clinicalgenome.org/) to assess and document the applicable rules for each variant. Provisional classifications made by the curators were comprehensively presented to the chairs and members of the HL VCEP during the monthly meetings. Members provided verbal feedback during these calls to help modify and approve the various ACMG/AMP codes as necessary. Monthly calls between the biocurators and the chairs of the group were utilized to finalize the classification of variants and to discuss the language of the classification summary text. The average curation time for an individual variant was 40 minutes, with a range from 10 minutes to 2 hours. The expert classifications of these variants were submitted to ClinVar on a quarterly basis (Fig. 1).

RESULTS

Curated variants

Since the specification and promulgation of the ACMG/AMP variant rules by the HL VCEP [5, 9], 157 variants—including the 51 variants used to pilot the newly specified rules—across nine HL genes (Supplementary Figure 1, Supplementary Table 1) underwent an exper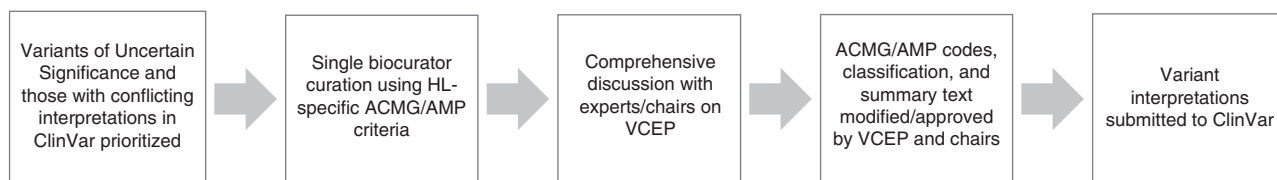t review and classification process as shown in Fig. 1. The majority of variants were missense ($n = 124$), while the remaining were in essential ($+/-1,2$) splice sites ($n = 4$), frameshift ($n = 7$), synonymous ($n = 5$), nonessential splice regions ($n = 10$), nonsense ($n = 3$), inframe deletion ($n = 1$), exon deletion ($n = 1$), start loss ($n = 1$), or in untranslated region (UTR) ($n = 1$) variants (Supplementary Table 1). As of 8 January 2021, 937 classifications have been submitted from 70 submitters for these 157 variants. Submitters were mainly affiliated with academic or commercial diagnostic laboratories, while a few were from academic research institutions.

Prior to expert curation, 100 variants had conflicting interpretations, 17 variants had VUS classifications, and 40 variants had no conflicts in ClinVar. Of the 100 variants with conflicting interpretations, 76 variants had medically significant conflicting classifications (P/LP vs. VUS/LB/B as defined by Harrison et al. [8]), and 24 variants had VUS versus LB/B conflicts. Only three variants had conflicting classifications of P/LP versus LB/B. For the 40 variants with no conflicting classifications, 33 variants were supported by multiple submissions, 5 variants had only one submission, and 2 variants had no previous submissions. Of these 40 variants, some were chosen to pilot the initial specifications of the rules while others were considered clinically significant variants or common pathogenic variants in a specific subpopulation [5] (Supplementary Figure 1).

Expert curation outcomes

After applying the HL-specific ACMG/AMP variant rules, 73 of the 117 variants (63%) with either conflicting or VUS classifications were classified as B, LB, LP, or P, while 44 variants (37%) were classified as VUS (Fig. 2). A significant proportion of the resolved classifications belonged to the VUS vs LB/B conflicting category where 20 of those 24 variants (83%) were classified as B or LB and the remaining as VUS. On the other hand, 38 of the 63 variants (60.3%) with VUS versus LP/P conflicting interpretations were classified as P or LP, one was classified as LB, and the remaining as VUS. Four of 17 variants (24%) with single or multiple VUS submissions in ClinVar were classified as B ($n = 1$) or LB ($n = 2$), or LP ($n = 1$) (Fig. 2a). Of the 40 non-VUS variants without conflicts, 13 (32.5%) were classified as B or LB, while 23 (57.5%) were classified as P or LP (Fig. 2a). Overall, 109 of the 157 variants (69%) were assigned non-VUS classifications (B, LB, LP, P) after applying the HL-specific ACMG/AMP modified variant interpretation guidelines by the expert group (Fig. 2b).

Criteria contribution to the final classification

In classifying the 157 variants, the most commonly applied criteria codes were PM2 (absence or rare occurrence in population databases) at two different strength levels (PM2_Supporting and PM2) and PM3 (allelic observations in recessive cases) at four different strength levels (PM3_Supporting, PM3, PM3_Strong, PM3_Very Strong), as specified by the HL VCEP [5]. Both the PM2 and PM3 codes were used 80 times and were applied to 77% (49/64) and 88% (56/64) of the variants classified as P/LP, respectively (Supplementary Figure 2). The in silico prediction code PP3, specified using REVEL [5, 14], was applied 64 times as
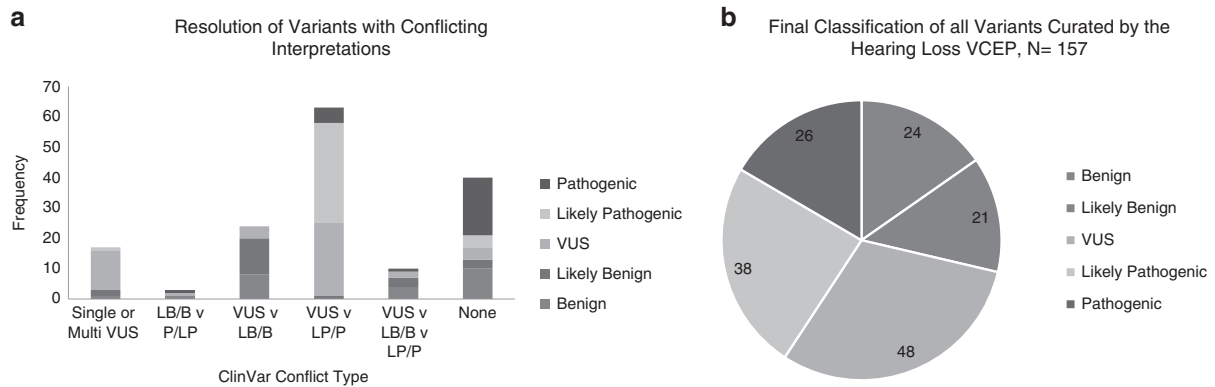
| Variants of Uncertain Significance and those with conflicting interpretations in ClinVar prioritized | → | Single biocurator curation using HL-specific ACMG/AMP criteria | → | Comprehensive discussion with experts/chairs on VCEP | → | ACMG/AMP codes, classification, and summary text modified/approved by VCEP and chairs | → | Variant interpretations submitted to ClinVar |

Fig. 1 Workflow highlighting the steps in the expert curation process to resolve conflicting interpretations or variants of uncertain significance. Refer to "Materials and Methods" for a detailed description of the curation process. ACMG/AMP American College of Medical Genetics and Genomics/Association for Molecular Pathology, HL hearing loss, VCEP Variant Curation Expert Panel.

a
Resolution of Variants with Conflicting Interpretations

b
Final Classification of all Variants Curated by the Hearing Loss VCEP, N= 157

Fig. 2 Outcomes of expert variant interpretation. (**a**) Resolution of variants with different conflict types into pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), or benign (B) by the ClinGen HL-EP. *y*-axis represents the number of variants; *x*-axis represents the conflict type in ClinVar. "Single" and "multi" refer to single and multiple submitters in ClinVar, respectively. (**b**) Final classifications of all variants curated by the Hearing Loss VCEP (*N* = 157).

the second most commonly used code, and was applied to 59% (38/64) of P/LP variants. The BA1, BS1, and BS1_Supporting allele frequency codes, as specified by the expert panel, were applied 59 times and to 91% (41/45) of the B/LB classifications. The phenotype code PP4 was used 47 times and was applied to 58% (37/64) of P/LP variants. The specified segregation code PP1 at three different strength levels (PP1, PP1_Moderate, PP1_Strong) was used 31 times and was applied to 41% (26/64) of P/LP variants. Finally, the specified functional codes (BS3_P, PS3_P, PS3_M, and PS3) [5] were used 31 times, 48% (15/31) of which were toward P/LP classifications. Several other codes were utilized as specified by the HL VCEP and as shown in Supplementary Figure 2.

DISCUSSION

Of the 28 ACMG/AMP criteria, 25 were specifically tailored to hereditary HL [5, 9]. The HL VCEP included an international group of scientists, clinicians, geneticists, genetic counselors, and laboratory directors. This panel leveraged the collective members' expertise and knowledge, along with internal unpublished data sets accessible to the group, to characterize several HL attributes such as prevalence, penetrance, genetic and allelic heterogeneity, and phenotypic presentations (onset, severity, frequencies affected, symmetry and audiometric profile) specific to several HL-associated genes. This information was then used to define the criteria for variant classification in hearing loss [5].

Our panel implemented these HL-specific guidelines to classify 157 variants. One hundred and nine variants (69%) were classified into non-VUS categories. Interestingly, the commonly used classification criteria codes were specified either for HL (e.g., PM2, BA1, BS1, BS1_Supporting, PP3, PP4) or, in collaboration with the ClinGen Sequence Variant Interpretation (SVI) workgroup, more broadly for recessive disorders (e.g., PM3) or any disorder (e.g., PP1). Application of criteria without disease-specific guidance is a significant contributor to conflicting classifications, emphasizing the importance of expert guidance and specification in usage of the ACMG/AMP rules. Several variants classified as P and LP also had BA1 and BS1 codes that were applied to them. Given that many of these variants were founder variants in specific subpopulations or had other evidence such as case–control data and case counts, the expert group decided to exclude the conflicting benign population frequency codes from the overall clinical interpretation of the variant.

Sharing of internal clinical laboratory case data among members of the HL VCEP has also served as an invaluable resource for resolving variants with conflicting interpretations. For example, the c.1708G>A (p.Val570Ile) variant in *SLC26A4*

previously had a conflicting classification and a medically significant classification difference of VUS vs LP. The HL VCEP resolved this conflict by counting 2 compound heterozygous internal cases from the Laboratory of Molecular Medicine, which led to the application of PM3_Strong, and PP4, leading to a LP classification. Without these internal cases, the variant would have been classified as VUS due to a lack of published case-level evidence.

Our curation process included bimonthly calls where biocurators first present all evidence used for each variant classification to the experts. Based on the feedback, biocurators often reached out to ClinVar submitters, authors of certain publications, and/or any of the experts to gather additional unpublished data required for final variant interpretation. The group also relied on feedback from expert clinicians regarding any genotype–phenotype correlations. The final evidence was then discussed on intervening monthly calls with the HL VCEP chairs, and if needed on subsequent calls with the larger group, to reach a final classification, including a text-based variant evidence summary documenting all applied evidence to be submitted to ClinVar and publicly accessible through ClinGen's Evidence Repository (https://erepo.clinicalgenome.org/evrepo/). This process has been, and continues to be, optimized for more efficient expert interpretations while still leveraging the diverse expertise within the group. It is important to note that this initial effort was mostly focused on variants with conflicting or uncertain significance to highlight the utility of the specified rules. In this process, a significant effort was dedicated to contacting ClinVar submitters and obtaining additional unpublished evidence to resolve interpretation discrepancies. We therefore expect this process to be faster for novel variants or ones without conflicting evidence. Ultimately, this process can be optimized to strike a balance between efficiency and leveraging the expertise of any disease group.

We have focused this pilot project on variants in nine HL-associated genes, *USH2A, SLC26A4, GJB2, MYO7A, CDH23, TECTA, COCH, KCNQ4,* and *MYO6,* for which ACMG/AMP specifications were tailored. However, most of those specified rules are equally applicable to other HL-associated genes. As such, the population frequency (BA1, BS1, PM2), allelic (PM3, BP2), de novo (PS2, PM6), segregation (PP1), predicted effects (PVS1, PM4, PP3, BP3, BP4, BP7), case–control (PS4), and their varied strength levels as specified by the expert group, are currently being extended to other genes. The group will also continue to prioritize curation of variants in HL genes with medically significant differences and those of uncertain significance.

In summary, our study shows that expert curation using disease-specific modifications of ACMG/AMP guidelines resolves discrepancies in variant classification, leading to more consistent

results for patients in need of accurate diagnoses and management decisions.

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