

Marina T. DiStefano, Madeline Y. Hughes, Mayher J. Patel, Emma H. Wilcox, and Andrea M. Oza*

Expert interpretation of genes and variants in hereditary hearing loss

<https://doi.org/10.1515/medgen-2020-2018>

Received January 21, 2020; accepted March 31, 2020

Abstract: Background: Hearing loss (HL) is the most common sensory deficit from birth, with at least 50 % due to an underlying genetic etiology. A genetic evaluation is a recommended component to the medical workup for HL, and a genetic diagnosis can impact medical management and provide prognostic and recurrence risk information. The accuracy of a genetic diagnosis relies on the evidence supporting the gene–disease relationship, as well as the evidence supporting individual variant classifications. As such, the ClinGen Hearing Loss Working Group was formed and tasked with curating genes associated with genetic hearing loss and developing specifications of the ACMG/AMP variant interpretation guidelines with the goal of improving the genetic diagnosis of patients with HL.

Objectives: To describe the prioritization and expert curation of genes and variants associated with HL performed under the purview of the ClinGen Hearing Loss Gene and Variant Expert Panels (HL GCEP and VCEP).

Materials and methods: HL genes were taken from clinical testing panels in the Genetic Testing Registry and prioritized based on a nonsyndromic presentation. Variants were taken from ClinVar and those with diverse data types and medically significant conflicts were prioritized to test the specified variant interpretation guidelines and to resolve classification discrepancies, respectively.

Conclusions: The ClinGen HL GCEP has curated 174 gene–disease pairs. The HL VCEP has submitted 77 variants, including the previously controversial p.Met34Thr and p.Val37Ile variants in *GJB2*, into ClinVar, as an FDA-recognized database. Collaboration across clinics and laboratories were crucial to these curations and highlight the impact that data sharing can have on patient care.

*Corresponding author: **Andrea M. Oza**, Laboratory for Molecular Medicine, Partners Healthcare Personalized Medicine, 65 Landsdowne Street Suite 350, Cambridge, MA, USA, e-mail: amoza@partners.org

Marina T. DiStefano, The Broad Institute of MIT and Harvard, Cambridge, MA, USA; and Laboratory for Molecular Medicine, Partners Healthcare Personalized Medicine, Cambridge, MA, USA

Madeline Y. Hughes, Mayher J. Patel, Emma H. Wilcox, The Broad Institute of MIT and Harvard, Cambridge, MA, USA

Keywords: hearing loss, gene curation, variant curation, genetics, ACMG/AMP variant interpretation guidelines

Introduction

Hearing loss is a common condition with a prevalence of 2 to 3 in 1000 newborns, with approximately 50 % of cases having a genetic etiology [1]. With the complexity of the auditory system, genetic hearing loss is also particularly heterogenous, with over 100 genes proposed to be associated with nonsyndromic hearing loss (NSHL) and over 400 genes proposed to be associated with syndromic hearing loss (Hereditary Hearing Loss, <http://hereditaryhearingloss.org>, [2]). Thus, it is a prime disease area requiring expert gene and variant curation. The Clinical Genome Resource (ClinGen, www.clinicalgenome.org) is an NIH-funded initiative building an authoritative central resource to define the clinical relevance of genes and variants for use in precision medicine and research [3]. The ClinGen Hearing Loss Clinical Domain Working Group (ClinGen HL CDWG, <https://tinyurl.com/HLCDWG>) was formed in the summer of 2016. The group has an international representation with >50 members from 26 institutions across five continents and varied expertise in research, genetic counseling, clinical genetics, and laboratory genetics. The goals of the HL CDWG are to curate genes proposed to be associated with genetic hearing loss (in the Gene Curation Expert Panel, HL GCEP) and to specify the ACMG/AMP guidelines for interpreting sequence variants in genes related to hearing loss (in the Variant Curation Expert Panel, HL VCEP) [4]. Prioritizing NSHL and syndromes that appear to be nonsyndromic with hearing loss as the presenting feature, the ClinGen GCEP curated 174 gene–disease pairs (Supplemental Table 1, <https://search.clinicalgenome.org/kb/gene-validity>) [5]. The group also specified ACMG/AMP sequence variant interpretation guidelines for nine hearing loss genes, and as of December 2019 has classified and submitted 77 variants to ClinVar as a three-star FDA-recognized expert panel (<https://www.ncbi.nlm.nih.gov/clinvar/submitters/506744/>) [6]. Multi-institutional expert collaboration such as this has contributed to signifi-

icant curation work in the genetic hearing loss field and has aimed to improve classification standardization and, in turn, patient care.

Gene curation

In an era where the cost of exome and genome sequencing is ever decreasing, gene curation, the evaluation of a gene and its relationship with disease, is critical for variant interpretation. The ACMG/AMP sequence variant interpretation guidelines state that care should be taken when interpreting variants in candidate genes that are not conclusively associated with disease and that variants in these “genes of uncertain significance” should always be classified as variants of uncertain significance if clinically reported [4]. Thus, without the curation of the clinical validity of a gene–disease relationship, variants cannot be classified. ClinGen has developed a semi-quantitative framework to classify the clinical validity of gene–disease pairs and ClinGen GCEPS use this framework to classify genes in their disease areas of interest as Definitive (12–18 points, replication over time), Strong (12–18 points), Moderate (7–11 points), Limited (1–6 points), Disputed, Refuted, and No Evidence [7]. This framework largely involves scoring published genetic evidence in the form of case-level, segregation, and case-control data and experimental evidence that can range from biochemical experiments to functional studies of transfected cells to animal models that recapitulate the disease [7]. The HL GCEP has curated 174 gene–disease pairs in 155 unique genes with 87 (50%) as Definitive, 12 (7%) as Strong, 25 (15%) as Moderate, 35 (20%) as Limited, 10 (6%) as Disputed, and 5 (3%) as Refuted (Figure 1a, Supplemental Table 1). All genes are curated in ClinGen’s gene curation interface so that a summary of scored evidence can be published directly to the ClinGen website (www.clinicalgenome.org) as soon as a curation is expert-approved.

Generating a gene list

The HL GCEP prioritized genes associated with NSHL, syndromic genes in which hearing loss is a presenting feature, and syndromic genes in which hearing loss is a feature; other syndromic features could be overlooked during clinical evaluation. To generate a gene list, all genes present on two or more next-generation sequencing panels (those with at least 20 genes) for hearing loss from 17 international and US-based laboratories were included in the

analysis. Those genes related to NSHL or for which hearing loss was a presenting feature were fully curated using the ClinGen framework. For genes related to syndromic conditions in which hearing loss was not a presenting feature, an abbreviated syndromic curation was performed which involved determining (1) if hearing loss is a diagnostic feature of the syndrome; (2) the penetrance of hearing loss in individuals with pathogenic variants in the gene; (3) the age of onset of hearing loss; (4) the severity, progression, and audiogram shape of the hearing loss (when available); and (5) if individuals with isolated hearing loss were evaluated to rule out the presence of other features of the syndrome [5]. In total, 152 genes were prioritized for curation in this way. Three additional genes were added to the list based on suggestions from the GCEP experts because of case observations in their laboratories or clinics.

Genes related to multiple conditions

Many genes were proposed to be associated with multiple conditions in the Online Mendelian Inheritance in Man (OMIM) database or the literature. If a gene on the prioritized list was related to multiple conditions, a precuration was performed to evaluate the mode of inheritance, the mechanism of disease, and phenotypic features of each condition to determine whether to lump the diseases into one curation record or to split them into individual records per the guidelines from the ClinGen Lumping and Splitting group (<https://clinicalgenome.org/working-groups/lumping-and-splitting/>). Additionally, 14 genes were associated with syndromic and nonsyndromic conditions. In these cases, the HL GCEP was cautious with phenotyping when scoring case-level information. For example, in the genes related to both Usher syndrome and NSHL (*ADGRV1*, *CDH23*, *CIB2*, *MYO7A*, *PCDH15*, *USH1C*, *USH1G*, *WHRN*), if thorough eye exams were not performed, if the age of an individual was not provided, or if the individual was too young to manifest vision problems, the case was not scored for either disease record. When possible, authors were contacted to clarify the phenotype in published cases before they were scored. Animal models were generally only scored for the syndromic or nonsyndromic disease recording, depending on the phenotype.

Clinical validity examples

In total, 174 gene–disease pairs in 155 unique genes with 87 (50%) scored as Definitive, 12 (7%) as Strong, 25 (15%) as Moderate, 35 (20%) as Limited, 10 (6%) as Disputed, and 5

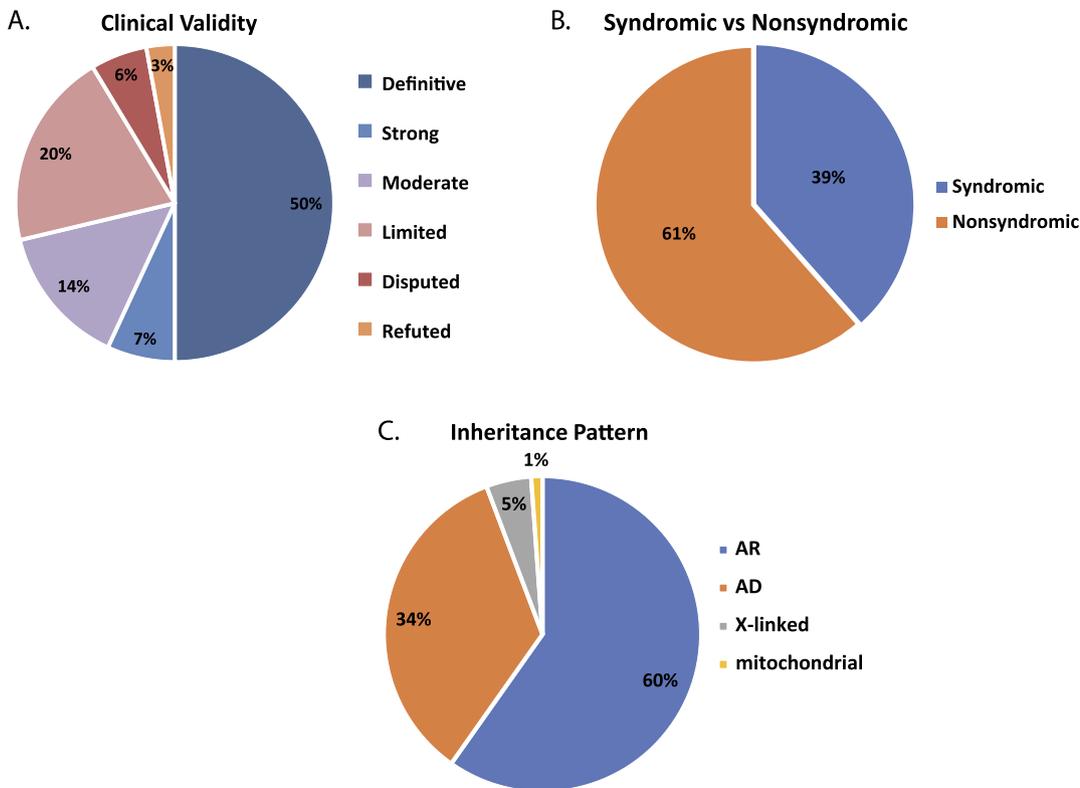


Figure 1: Clinical validity results. a The clinical validity of 174 gene–disease pairs: Definitive = 87, Strong = 12, Moderate = 25, Limited = 35, Disputed = 10, Refuted = 5. b Syndromic ($n = 67$) and nonsyndromic ($n = 107$) breakdown of 174 gene–disease pairs. c Curations split by inheritance pattern: autosomal recessive (AR) = 104, autosomal dominant (AD) = 60, X-linked = 8, mitochondrial = 2.

(3%) as Refuted (Figure 1a, Supplemental Table 1) were curated. Based on the way the gene list was generated, these were largely nonsyndromic curations (107, 61%) instead of syndromic (67, 39%) curations (Figure 1b) and when separated by inheritance pattern, 104 (60%) were autosomal recessive (AR), 60 (34%) autosomal dominant, 8 (5%) X-linked, and 2 (1%) mitochondrial (Figure 1c). Evidence for all curations is provided on the ClinGen website; only a few representative examples of curations are included below.

WHRN and autosomal recessive Usher syndrome Type 1, Definitive (approved 5/10/2017)

WHRN was proposed to be related to AR Usher syndrome Type 1 in numerous publications. Genetic evidence was maximized to 12 points by scoring seven individuals with Usher syndrome who were compound heterozygous for loss of function variants and maximizing segregation evidence by scoring segregations in four families [8–12]. For experimental evidence, the whirler mouse model that had hearing loss and vestibular dysfunction and the rescue of the phenotype by adding exogenous *WHRN* were each scored 2 points [13]. Expression experiments demonstrating that *WHRN* is expressed in the murine inner ear

and retina and protein interaction yeast-2-hybrid experiments demonstrating that *WHRN* physically interacts with *USH2A*, a known Usher syndrome gene, scored a total of 2 points and maximized the experimental evidence at 6 points. Because the first publication was published in 2002, this gene–disease pair was replicated over time (>2 publications with human variants over 3 years) and classified as Definitive.

BDP1 and AR NSHL, Limited (approved 6/13/18)

BDP1 was proposed to be related to AR NSHL [14]. One stop-loss variant (NM_018429.2:c.7873T>G [p.Ter2625Glu]) was identified in the homozygous state in four members of a family of Qatari descent with bilateral, sensorineural, postlingual onset (ages 2–4 years) progressive hearing loss. This variant was scored 0.5 points and 2 segregation points were awarded considering the four affected and four unaffected family members. For experimental evidence, expression experiments demonstrating that *BDP1* is expressed in murine endothelial cells of stria vascularis capillaries and mesenchyme-derived cells and surrounding extracellular matrix around the cochlear duct, including the spiral ligament and basilar membrane, were

awarded 0.5 points in total. As only one family was identified with a large linkage interval segregating with hearing loss and the experimental evidence does not directly demonstrate that *BDP1* is required for cochlear function, this gene–disease pair scored 3 points, which corresponds to a Limited classification.

Variant interpretation

A genetic consult, including genetic testing, is a recommended part of the clinical evaluation for hearing loss, as identifying a genetic etiology can help distinguish between syndromic and nonsyndromic forms and provide prognostic and recurrence risk information [1]. The accurate interpretation of sequence variants is essential to providing a genetic diagnosis. Inconsistencies and discrepancies in variant interpretation have been well documented and can have serious implications for patient care [15–18]. While the ACMG/AMP guidelines on the interpretation of sequence variants provide a process for classifying variants, many parts of the evidence framework lack specificity and are therefore open to interpretation. The ClinGen HL VCEP was formed to further specify parts of the ACMG/AMP guidelines to provide guidance, reduce inconsistencies in variant classification, and resolve discrepancies.

Between August 2016 and September 2018, the HL VCEP developed specifications of the ACMG/AMP variant

interpretation guidelines. The presence of genetic heterogeneity, common founder variants, multiple inheritance patterns, and involvement of genetic syndromes posed unique challenges to this process. Since the development of these specifications, the HL VCEP has been providing expert classification of sequence variants with discrepancies in ClinVar. As of December 2019, 77 variants curated by the HL VCEP are present in ClinVar as a three-star FDA-recognized expert panel (Figure 2, <https://www.ncbi.nlm.nih.gov/clinvar/submitters/506744/>) [6]. Here we review key considerations for interpreting sequence variants for hearing loss.

Minor allele frequency thresholds

The standardization of minor allele frequency (MAF) thresholds for a benign or likely benign classification is essential for accurate and consistent variant interpretation. The threshold must be set at a high enough frequency so that common pathogenic variants are not disregarded or filtered out, but it cannot be so high that genetic testing reports are overburdened with variants of uncertain significance that are unlikely to be disease causing.

Several publications have described methods for setting MAF thresholds for classifying benign hearing loss variants [6, 19, 20]. Despite differing approaches, the MAF thresholds have been similar, with recommended MAF thresholds for a benign classification ranging from 0.005 to 0.006 [6, 19, 20]. The HL VCEP recommends using MAF

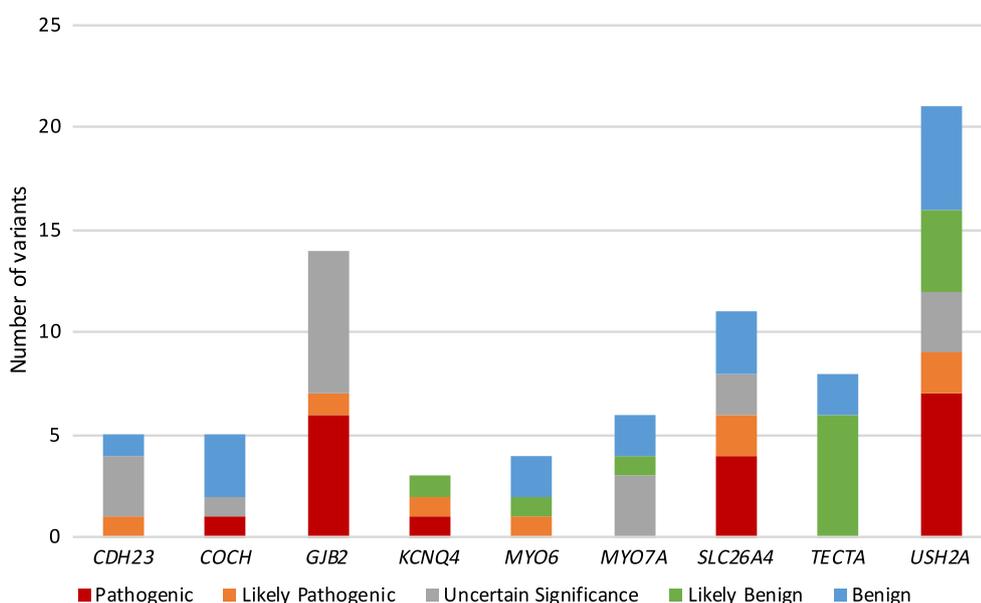


Figure 2: HL VCEP curated variants in ClinVar. A total of 77 variants curated by the HL VCEP have been uploaded into ClinVar, shown here with the number of variants in each gene and classification.

Table 1: List of variants which should be excluded from benign classification based on allele frequency.

Gene	cDNA	Protein	Pathogenicity	MAF* % (Population)
<i>GJB2</i>	c.-22-2A>C		Uncertain significance	0.4 % (Ashkenazi Jewish)
<i>GJB2</i>	c.34G>T	p.Gly12Cys	Likely pathogenic	0.3 % (Latino)
<i>GJB2</i>	c.35delG	p.Gly12Valfs*2	Pathogenic	0.9 % (Non-Finnish European)
<i>GJB2</i>	c.71G>A	p.Trp24*	Pathogenic	0.4 % (South Asian)
<i>GJB2</i>	c.101T>C	p.Met34Thr	Pathogenic	2.0 % (Finnish)
<i>GJB2</i>	c.109G>A	p.Val37Ile	Pathogenic	8.0 % (East Asian)
<i>GJB2</i>	c.167delT	p.Leu56Argfs*26	Pathogenic	1.6 % (Ashkenazi Jewish)
<i>GJB2</i>	c.235delC	p.Leu79Cysfs*3	Pathogenic	0.6 % (East Asian)
<i>SLC26A4</i>	c.349C>T	p.Leu117Phe	Pathogenic	0.5 % (Ashkenazi Jewish)
<i>SLC26A4</i>	c.919-2A>G	p.?	Pathogenic	0.5 % (East Asian)

*The highest subpopulation frequency in the Genome Aggregation Database (gnomAD) is shown. *GJB2* (NM_004004.5), *SLC26A4* (NM_000441.1).

thresholds of 0.005 for benign criteria (BA1) and 0.003 for likely benign criteria (BS1).

However, applying these thresholds still requires preserving high frequency pathogenic variants, as there are several variants in *GJB2* and *SLC26A4* that exceed these cutoffs. The HL VCEP provides a list of variants that should be excluded from any automated classification that utilizes MAF alone (Table 1).

Lastly, when evaluating the MAF of a variant, it is recommended to utilize a filtering allele frequency, which determines the “maximum tolerated allele count,” based on the size of the population and at a specified confidence level (<http://cardiodb.org/allelefrequencyapp/>). This prevents inaccurate interpretation of an MAF from a small population cohort.

p.Met34Thr and p.Val37Ile in *GJB2*

Variation in the *GJB2* gene accounts for the most commonly identified cause of AR NSHL [21, 22]. It is allelically heterogeneous, with over 100 pathogenic and likely pathogenic variants reported in ClinVar. However, there are also common founder variants across several different populations, each with a high allele frequency which would meet the MAF thresholds described above (Table 1).

The interpretation of two common variants in *GJB2* (NM_004004.6), c.109G>A (p.Val37Ile) and c.101T>C (p.Met34Thr), has been controversial due to their significantly high MAFs and reports in homozygous and compound heterozygous individuals with normal hearing [21, 23]. In 2019, The HL VCEP published consensus classification for these two variants. Data were collected from 15 laboratories and clinics. Case-control comparisons were performed and the ACMG/AMP guidelines were applied [24]. Homozygotes for c.109G>A (p.Val37Ile) and c.101T>C

(p.Met34Thr) were significantly enriched in cases, with odds ratios of 16 (95 % CI, 11–25, $Z = 13$, $P < 0.0001$) and 20 (95 % CI 17–24, $Z = 31$, $P < 0.0001$), respectively [24]. These variants were associated with mild to moderate hearing loss, with potentially reduced penetrance, consistent with findings from previous publications [21–24]. Additional data utilized in the classification of these variants included segregation data and functional evidence. As such, these variants were submitted to ClinVar by the HL VCEP as pathogenic for AR hearing loss (Variation IDs 17000 and 17023). It is intended that the classification and evidence summaries for these variants will help to reduce conflicts in classification amongst clinical laboratories.

Allelic (in trans) data – PM3

Observing that a novel variant is in *trans* with a known pathogenic variant can be used as evidence of pathogenicity for recessive diseases such as AR NSHL. Often this requires testing the parents of the affected individual to determine phase. The ACMG/AMP guidelines state that this information can be used as moderate evidence of pathogenicity, and if there are multiple observations, the strength of the evidence can be increased. However, the guidelines did not state how many observations are required to meet a strong or very strong level of evidence. Furthermore, it was not stated whether homozygous occurrences or the observation of two rare novel variants could be counted as evidence. As such, the HL VCEP and the SVI developed precise recommendations on how to score this evidence [6].

One further consideration when using allelic data as evidence is the allele frequency of the known pathogenic variant with which the variant in question is in *trans*. For example, the likelihood that a variant would be observed

in *trans* with a variant such as the incredibly common c.109G>A (p.Val37Ile) variant in *GJB2* (NM_004004.5) is much higher than for a variant that is absent or has a very low frequency in the general population. Caution should be used in this scenario, and the HL VCEP plans on making further recommendations on how to account for this, given the number of high frequency pathogenic variants that are causative for hearing loss.

Phenotypic data

Hearing loss phenotypic data include the age of onset, severity, laterality, presence of progression, and audiogram shape. Additional features such as exposure to aminoglycosides, temporal bone abnormalities, and clinical findings that may be part of a syndrome (e. g., retinitis pigmentosa in Usher syndrome) can also point towards a specific condition or gene.

The ACMG/AMP guidelines consider phenotypic information as a supporting piece of evidence towards pathogenicity when that phenotype is highly specific for the gene in which the variant was identified (PP4 code) [4]. Due to significant heterogeneity, audiometric features of NSHL cannot be used as evidence. However, the HL VCEP developed a list of phenotypes for which PP4 can be applied. The list primarily includes genetic syndromes or distinct features. An example is hearing loss with enlarged vestibular aqueducts, which is observed in patients with pathogenic *SLC26A4* variants.

Variant interpretation can be particularly challenging for genes which are associated to both nonsyndromic and syndromic hearing loss. An important first step is determining whether there is truly enough evidence to support a nonsyndromic form, as discussed in the Gene Curation section above. The HL GCEP has curated several genes in which variants have been associated in the literature with both nonsyndromic and syndromic hearing loss, including *ACTG1*, *ADGRV1*, *CDH23*, *MYO7A*, *PCDH15*, *USH1G*, *USH1C*, and *WFS1*. While a gene may be associated with both nonsyndromic and syndromic hearing loss, the evidence available for an individual variant may not be sufficient to make the distinction. Here we present an example: A missense variant in *CDH23* with enough evidence for a likely pathogenic classification has only been described in individuals too young to determine or rule out the presence of retinitis pigmentosa. The evidence supports that it is likely pathogenic for hearing loss, but it cannot be determined with certainty whether the variant causes Usher syndrome or true NSHL.

Data sharing improves genetic interpretation

Lastly, the hearing loss expert panels have found significant value in the collaboration between clinical and research laboratories and clinical centers. Collaboration is an integral part of efforts across ClinGen and ClinVar. There were three gene–disease pairs in the HL GCEP curation list that would have been underscored without collaborative data. *OTOG*, *GRHL2*, and *ESRRB* all would have been classified as Moderate by scoring the published literature alone; however, with further case-level information from ClinVar submitters who had submitted variant classifications in these genes, *OTOG* and *ESRRB* were classified as Definitive and *GRHL2* was classified as Strong. In addition, the sharing of internal data has significantly impacted variant classification. For example, internal segregation data shared by the Tokyo Medical Center resulted in the reclassification of NM_000260.4(MYO7A):c.2558G>A (p.Arg853His) to likely pathogenic. Without this evidence, the variant would have been classified as uncertain significance. These examples underscore the importance of data sharing and depositing variants in ClinVar.

Conclusion

With the complexity of the auditory system, hearing loss is a particularly genetically heterogeneous condition. The ClinGen HL CDWG was formed in July 2016 as a group of experts with diverse specialties to tackle gene and variant curation. Through this curation work, 174 gene–disease pairs have been curated and published on the ClinGen website, ACMG/AMP guidelines have been specified for the most common nine hearing loss genes, and 77 variants have been deposited in ClinVar as expert panel curations. Expert curation such as this helps to improve genetic interpretation and, in turn, patient care.

Patients' Rights and Animal Protection Statement: This article does not contain any studies with human or animal subjects.

Disclosure: All authors are curators for the Hearing Loss Gene Curation and Variant Curation Expert Panels within ClinGen. The following authors are an employee, trainee, or consultant for a fee-for-service laboratory that offers hearing loss panel testing: Marina T. DiStefano, Andrea M. Oza.

References

- [1] Alford RL, Arnos KS, Fox M et al.. American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss. *Genet Med.* 2014;16:347–55.
- [2] Abou Tayoun AN, Al Turki SH, Oza AM et al.. Improving hearing loss gene testing: a systematic review of gene evidence toward more efficient next-generation sequencing-based diagnostic testing and interpretation. *Genet Med.* 2016;18:545–53.
- [3] Rehm HL, Berg JS, Brooks LD et al.. ClinGen—the Clinical Genome Resource. *N Engl J Med.* 2015;372:2235–42.
- [4] Richards S, Aziz N, Bale S et al.. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405–24.
- [5] DiStefano MT, Hemphill SE, Oza AM et al.. ClinGen expert clinical validity curation of 164 hearing loss gene-disease pairs. *Genet Med.* 2019;21:2239–47.
- [6] Oza AM, DiStefano MT, Hemphill SE et al.. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. *Human Mutat.* 2018;39:1593–613.
- [7] Strande NT, Riggs ER, Buchanan AH et al.. Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. *Am J Hum Genet.* 2017;100:895–906.
- [8] Audo I, Bujakowska K, Mohand-Said S et al.. A novel DFNB31 mutation associated with Usher type 2 syndrome showing variable degrees of auditory loss in a consanguineous Portuguese family. *Mol Vis.* 2011;17:1598–606.
- [9] Besnard T, Vache C, Baux D et al.. Non-USH2A mutations in USH2 patients. *Human Mutat.* 2012;33:504–10.
- [10] Ebermann I, Scholl HP, Charbel Issa P et al.. A novel gene for Usher syndrome type 2: mutations in the long isoform of whirlin are associated with retinitis pigmentosa and sensorineural hearing loss. *Hum Genet.* 2007;121:203–11.
- [11] Jiang L, Liang X, Li Y et al.. Comprehensive molecular diagnosis of 67 Chinese Usher syndrome probands: high rate of ethnicity specific mutations in Chinese USH patients. *Orphanet J Rare Dis.* 2015;10:110.
- [12] Wang X, Feng Y, Li J et al.. Retinal Diseases Caused by Mutations in Genes Not Specifically Associated with the Clinical Diagnosis. *PLoS ONE.* 2016;11:e0165405.
- [13] Mburu P, Mustapha M, Varela A et al.. Defects in whirlin, a PDZ domain molecule involved in stereocilia elongation, cause deafness in the whirler mouse and families with DFNB31. *Nat Genet.* 2003;34:421–8.
- [14] Giroto G, Abdulhadi K, Buniello A et al.. Linkage study and exome sequencing identify a BDP1 mutation associated with hereditary hearing loss. *PLoS ONE.* 2013;8:e80323.
- [15] Amendola LM, Jarvik GP, Leo MC et al.. Performance of ACMG-AMP Variant-Interpretation Guidelines among Nine Laboratories in the Clinical Sequencing Exploratory Research Consortium. *Am J Hum Genet.* 2016;99:247.
- [16] Booth KT, Azaiez H, Kahrizi K et al.. PDZD7 and hearing loss: More than just a modifier. *Am J Med Genet, Part A.* 2015;167A:2957–65.
- [17] Booth KT, Kahrizi K, Babanejad M et al.. Variants in CIB2 cause DFNB48 and not USH1J. *Clin Genet.* 2018;93:812–21.
- [18] Harrison SM, Dolinsky JS, Knight-Johnson AE et al.. Clinical laboratories collaborate to resolve differences in variant interpretations submitted to ClinVar. *Genet Med.* 2017;19:1096–104.
- [19] Rim JH, Lee JS, Jung J et al.. Systematic evaluation of gene variants linked to hearing loss based on allele frequency threshold and filtering allele frequency. *Sci Rep.* 2019;9:4583.
- [20] Shearer AE, Eppsteiner RW, Booth KT et al.. Utilizing ethnic-specific differences in minor allele frequency to recategorize reported pathogenic deafness variants. *Am J Hum Genet.* 2014;95:445–53.
- [21] Kenna MA, Feldman HA, Neault MW et al.. Audiologic phenotype and progression in GJB2 (Connexin 26) hearing loss. *Arch Otolaryngol Head Neck Surg.* 2010;136:81–7.
- [22] Snoeckx RL, Huygen PL, Feldmann D et al.. GJB2 mutations and degree of hearing loss: a multicenter study. *Am J Hum Genet.* 2005;77:945–57.
- [23] Pollak A, Skorka A, Mueller-Malesinska M et al.. M34T and V37I mutations in GJB2 associated hearing impairment: evidence for pathogenicity and reduced penetrance. *Am J Med Genet, Part A.* 2007;143A:2534–43.
- [24] Shen J, Oza AM, Del Castillo I et al.. Consensus interpretation of the p.Met34Thr and p.Val37Ile variants in GJB2 by the ClinGen Hearing Loss Expert Panel. *Genet Med.* 2019;21:2442–52.

Supplemental Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/medgen-2020-2018>).

Marina T. DiStefano

The Broad Institute of MIT and Harvard, Cambridge, MA, USA
Laboratory for Molecular Medicine, Partners Healthcare
Personalized Medicine, Cambridge, MA, USA

Madeline Y. Hughes

The Broad Institute of MIT and Harvard, Cambridge, MA, USA

Mayher J. Patel

The Broad Institute of MIT and Harvard, Cambridge, MA, USA

Emma H. Wilcox

The Broad Institute of MIT and Harvard, Cambridge, MA, USA

Andrea M. Oza

Laboratory for Molecular Medicine, Partners Healthcare
Personalized Medicine, 65 Landsdowne Street Suite 350,
Cambridge, MA, USA
amoza@partners.org