Journal Pre-proof

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ClinGen recuration of hearing loss associated-genes demonstrates significant changes in gene-disease validity over time

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Abstract

Purpose: The ClinGen Hearing Loss Gene Curation Expert Panel (GCEP) was assembled in 2016 and has since curated 174 gene-disease relationships (GDRs) using ClinGen's semi-quantitative framework. ClinGen mandates timely recuration of all GDRs classified as *Disputed*, *Limited*, *Moderate*, and *Strong*, every 2–3 years.

Methods: Thirty-five GDRs met the criteria for recuration within two years of original curation. Previous evidence was reevaluated using the latest curation guidelines and a comprehensive literature review was performed for new evidence. The recurations were approved by the GCEP and published to the ClinGen website (www.clinicalgenome.org).

Results: Eight out of 35 (22%) GDRs changed classification. Two *Moderate* and five *Strong* GDRs upgraded to *Definitive* due to new case evidence. One *Strong* was subsumed under another *Definitive* GDR, after evaluation of lumping/splitting of disease entities. Twenty-seven out of 35 remained unchanged with little to no new evidence reported.

Conclusion: Genes classified as *Moderate* and *Strong* are likely to build evidence and change in classification over time, whereas *Limited* are unlikely to gain evidence. These findings also highlight the critical role of recuration in ensuring that genetic tests and research studies incorporate the most up-to-date evidence into their efforts.

Keywords: ClinGen; deafness; Gene curation; genetic diagnosis; hearing loss.

Introduction

The Clinical Genome Resource (ClinGen)[1] is an authoritative central resource that defines the clinical relevance of genes and variants for use in precision medicine and research (www.clinicalgenome.org). ClinGen established an international curation ecosystem of experts[2] called Expert Panels (EPs) tasked with evaluating gene-disease relationships (GDRs) and variant pathogenicity in various disease areas. Using a common framework developed by the ClinGen Gene Curation Working Group (GCWG), the Gene Curation Expert Panels (GCEPs) curate the strength of evidence supporting or refuting a GDR in their respective fields.[1] This framework involves the curation of genetic and experimental evidence from published literature and other publicly available databases (e.g., ClinVar) and scoring them according to ClinGen's Standard Operating Procedures (SOP) to assign a clinical validity classification for the GDR (*Definitive, Strong, Moderate, Limited, Disputed, Refuted,* or *No known disease relationship*).

The Hearing Loss (HL) GCEP was one of the first GCEPs to be approved in 2016, and, for the first round of curation, completed curations of 164 GDR using ClinGen's semi-quantitative framework.[3,4] However, these gene-disease validity classifications are subject to change as ClinGen GCEPs primarily score peer-reviewed literature, whereby evidence is constantly accumulating as new cases and experimental data on the GDR are published. For accurate interpretation and diagnostic workup, it is crucial that ClinGen's curation reflects the latest developments in GDRs, as well as redefined disease entities with lumping and splitting conditions.[5] Moreover, curations are performed using versions of ClinGen's Gene Curation SOP, which are refined over time, potentially leading to changes in classifications. To keep the curations up-to-date, ClinGen developed recommendations and standard procedures for routine recuration of previously approved gene-disease validity classifications.[6] This approach is

essential to optimize the clinical sensitivity of diagnostic testing, reduce the rate of variants of uncertain significance generated by genes not implicated in disease, and avoid possible misdiagnoses due to erroneous gene-disease implications.[7] With a large gene list, the HL GCEP began its recuration efforts, using the latest guidelines, with recuration of 35 GDRs completed so far. The results of this recuration effort may inform and guide future recuration initiatives and guidelines for all ClinGen GCEPs.

Methods

Recuration gene selection

Per the ClinGen recuration guidelines (https://www.clinicalgenome.org/docs/gene-disease-validity-recuration-process/), *Moderate* GDRs require recuration every 2 years, while *Disputed*, *Limited*, or *Strong* relationships require recuration every 3 years. Recuration for *Definitive*, *Refuted*, or *No known disease relationship* is not necessary in the absence of new evidence supporting/contradicting the GDR. Of the 164 genes previously curated by the HL GCEP between 2017–2019[2], 35 genes met criteria for recuration between 2020–2024.

Recuration and expert review

Recuration of flagged genes was performed by a single curator using the most recent ClinGen framework SOP available at the time of recuration (ClinGen SOP v8–10) (https://clinicalgenome.org/docs/gene-disease-validity-standard-operating-procedure/). For each gene, all prior genetic and experimental evidence was reevaluated per the current ClinGen SOP, followed by assessment of any new published data. Genes with changes to their classification were presented on the GCEP monthly calls and reviewed by the GCEP for approval of the new classification. Genes with no new information were also presented to the GCEP for re-approval

of their initial classification, albeit in an expedited workflow. Once approved, all evidence was updated in the current release of the Gene Curation Interface (GCI) and the evidence summaries were edited to describe the changes in evidence after recuration.[8] The recurations were approved and are available on the ClinGen website (https://search.clinicalgenome.org/kb/affiliate/10007).

Results

Recuration gene list

The HL GCEP has curated 10 new GDRs since the first round of curation[3], totaling to 174 GDRs (n=97 *Definitive*, n=7 *Strong*, n=18 *Moderate*, n=36 *Limited*, n=12 *Disputed*, n=4 *Refuted*) between 2017–2023 (Supplemental Figure 1, Supplemental Table 1). Of these, 35 GDRs met criteria for recuration between 2020–2024, including 7 *Strong*, 9 *Moderate*, 17 *Limited*, and 2 *Disputed* GDRs (Table. 1). A total of 27 genes were associated with autosomal recessive (AR) (n=13), autosomal dominant (AD) (n=13) or X-linked (n=1) nonsyndromic hearing loss (NSHL), while 8 genes were associated with AR (n=6) or AD (n=2) syndromic hearing loss.

Recuration and reclassification

The 35 GDRs were recurated and reclassified based on the latest ClinGen clinical-validity assessment at the time of recuration. The recent SOPs (v8–10) include significant changes in scoring approaches for *de novo* variants, *in trans* occurrences, and ability to include online database/clinical lab data. Eight GDRs (22%) were reclassified based on new evidence (n=7) or due to scoring adjustments in the updated SOP (n=1) (Table. 1). Recurations mostly led to upgrades from *Moderate* (n=2) and *Strong* (n=5) to *Definitive*. One *Strong* GDR, *WFS1* (HGNC:12762)/AD neonatal diabetes, congenital sensorineural hearing loss and congenital

cataracts, was lumped under another *Definitive* GDR (*WFS1*/AD Wolfram-like syndrome), described in detail below. On the other hand, 27 GDRs retained their classification, including *Moderate* (n=7), *Strong* (n=1), and all *Disputed* (n=2) and *Limited* (n=17) genes (Figure 1).

*(Insert **Figure 1** here)*

***Moderate* to *Definitive* reclassifications**

Two *Moderate* classifications, *MSRB3* (HGNC:27375)/AR-nonsyndromic genetic deafness and *COL9A3* (HGNC:2219)/AR-Stickler syndrome, were reclassified as *Definitive* (Table. 1). Both pairs had new genetic and functional data to support their relationships, including animal models consistent with the disease and inheritance pattern (https://search.clinicalgenome.org/CCID:005437, https://search.clinicalgenome.org/CCID:004544).

As an example, *MSRB3* was originally curated in 2017 for autosomal recessive hearing loss and was classified as *Moderate* despite a clinical-validity score of 11.5 (*Moderate* range 7–11), on account of the limited number of causative variants reported in affected individuals. The initial curation was supported by only one publication,[9] which reported two unique variants segregating in eight different Pakistani families; however, both variants were present in the homozygous state in families with significant consanguinity. One of the variants was a nonsense variant (NC_000012.12:g.65308634C>T (Chr12, GRCh38): NM_001031679.3:c.55C>T p.(Arg19Ter)) and the other was a missense (NC_000012.12:g.65328584T>G (Chr12, GRCh38): NM_001031679.3:c.244T>G p.(Cys82Gly)) with *in vitro* functional evidence suggestive of a loss of function effect. Per the clinical-validity workflow/SOP, the genetic evidence presented in this report would reach a score of 6. With an additional 5.5 score from experimental evidence supporting the GDR, the total score was 11.5 which would theoretically be a *Strong* (*Strong* range 12–18) clinical validity classification. However, the HL GCEP downgraded the final classification to *Moderate* since the genetic evidence was from a single publication with a highly consanguineous study cohort. Upon recuration in 2021, a second hearing loss study in consanguineous Pakistani families reported the p.(Cys82Gly) variant in 5 families, and also

reported a novel NM_001031679.3:c.391-1G>A splice variant (NC_000012.12:g.65463154G>A (Chr12, GRCh38)) in one family.[10] Moreover, an internal case with another novel splice site variant (NC_000012.12:g.65368997G>A (Chr12, GRCh38): NM_001031679.3:c.264-1G>A) was reported by Laboratory for Molecular Medicine, Mass General Brigham Personalized Medicine (ClinVar Accession: SCV000271241.2). In reevaluating the GDR with this new evidence, the genetic score increased from 6 to 8.4, while the experimental score remained the same, bringing the total score to 13.9 points (*Definitive* range 12–18). With this new evidence, and replication in the literature, the classification of *MSRB3* was upgraded to *Definitive*.

***Strong* to *Definitive* reclassifications**

Of the seven *Strong* genes, five were upgraded to *Definitive* based upon identification of new cases reported in publications (Table. 1). *CDC14A* (HGNC:1718), associated with autosomal recessive hearing loss and male infertility syndrome, was the only gene which remained at *Strong*. All the genetic evidence scored for this GDR were from a single publication and with no new evidence published in the literature, the GCEP retained its classification at *Strong*.

Lumping vs Splitting reevaluation

The *WFS1*/AD neonatal diabetes, congenital sensorineural hearing loss and congenital cataracts GDR had the most substantial change in curation. This GDR was initially classified as *Strong* with a score of 12 points (*Strong* range 12–18) per SOP v7 in 2018. Simply reevaluating the evidence per SOP v9 decreased the score to 3.6 points due to changes in scoring of *de novo* variants, which falls in the *Limited* classification range (*Limited* range 1–6). This drastic decrease in scores warranted the GCEP to re-evaluate the validity of the disease assertions associated with *WFS1*. Initially, *WFS1* was curated for 3 disease entities (AR Wolfram syndrome, AD Wolfram-

like syndrome, and AD neonatal diabetes, congenital sensorineural hearing loss and congenital cataracts) in 2018 prior to the development of the Lumping and Splitting guidelines. In 2022, ClinGen published the Lumping and Splitting guidelines to help categorize and define the disease entity before evaluating the strength of the GDR for genes associated with multiple diseases or broad phenotypic spectra.[5] Based on criteria such as preexistent assertions, molecular mechanism, phenotypic variability, and inheritance pattern, a gene could either be curated for a single condition encompassing all phenotypes associated with the gene (lump) or separate conditions with distinct differences (split). Based on these guidelines, the GCEP lumped AD neonatal diabetes, congenital sensorineural hearing loss and congenital cataracts under AD Wolfram-like syndrome, due to similarities in inheritance pattern, phenotypic spectrum, and molecular mechanisms, and its evidence was included in the *WFS1*/AD Wolfram-like syndrome GDR which was classified as *Definitive* in 2018. The GCEP also curated the *WFS1* gene for AD NSHL, which was a new disease entity that was not considered in the initial curation. This recuration of the *WFS1* gene highlights the importance of considering the disease entity upon which curation is performed and the lumping/splitting guidance provided by ClinGen.

*(Insert **Table 1** here)*

Discussion

As of August 2024, the ClinGen HL GCEP has recurated 35 of 174 total GDRs, of which 22% (8/35) changed classification. Recuration not only led to changes in classification, but also to changes in lumping/splitting of disease entities for one gene. Across the eight genes that changed their classification, new evidence led to an upgrade in scoring, while the downgrade in scoring was largely due to re-evaluation of the existing evidence using updated ClinGen gene curation guidelines which have been adjusted to be more stringent over the years. The GDRs that maintained their classifications were mostly due to the lack of new evidence to score. Interestingly, no changes in classification were observed in the *Limited* or *Disputed* category, while many of the *Strong* and *Moderate* categories underwent upgrades. The observed trends over time may help refine the ClinGen recuration guidelines. Generally, GDRs that are initially scored in the lower range of the *Limited* category tend to remain unchanged over time. The lack of changes in the *Limited* category might suggest these genes are more likely to be falsely implicated in disease and therefore unlikely to accumulate further evidence. To distinguish whether this is the case, it is critically important for groups evaluating these genes to publish and/or submit new evidence to ClinVar. Going forward, the HL GCEP will reconsider evidence for *Limited* genes and decide if the GDR should be recurated as *Disputed* or even *Refuted* if the original evidence was insufficient to ever implicate the gene in disease. In this case, the submission of contradictory evidence or benign variants, which may not be included in peer-reviewed literature, is also very important.

Gene recuration is an ongoing effort across all ClinGen GCEPs, with expert-reviewed classifications being posted regularly. GCEPs across ClinGen hold monthly meetings where

experts review an average of 2-3 gene curations per meeting. Despite the limited GCEP capacity, it is crucial that these panels make every effort to keep up with the growing number of gene recurations while curating new GDRs as well. The high rate of classification changes of the hearing loss-associated genes in this study further emphasizes the critical need for continuous recuration efforts to ensure genetic tests and research apply the most up-to-date evidence in their diagnoses and studies.

Data Availability

All expert panel curations of the gene-disease relationships, and supporting evidence are publicly accessible from the Clinical Genome Resource website at www.clinicalgenome.org. The gene-disease clinical validity classifications are updated on an ongoing basis to reflect the most up-to-date evaluations.

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Author Contributions

Conceptualization: K.C.T., M.T.D., S.S.A.; Data curation: K.C.T., Formal analysis: K.C.T., M.T.D., S.S.A.; Funding acquisition: H.L.R.; Investigation: K.C.T., M.T.D., S.S.A.; Writing – original draft: K.C.T.; Writing – review & editing: M.T.D., S.S.A., A.N.A., A.M.O., P.A., R.W., E.E., E.B., J.R., V.G., A.A., J.A., V.A., D.B., H.A., K.TA.B., P.I.B., C.C., V.D., I.C., M.A.M., H.D., B.D., R.F., M.K., M.A.L., M.L., Y.L., R.M., T.M., K.N., A.P., S.R., I.R., L.A.S., I.S., S.S., J.S., B.V., R.J.S., H.L.R.

Ethics Declaration

This study is not considered human or animal subject research.

Conflict of Interest

Many authors are compensated clinical service providers as noted by their affiliations in health care systems or commercial genetic testing laboratories. The authors declare no other relevant conflicts of interest.

Supplemental file listing

Supplemental Figure 1: Gene curation progress of the HL GCEP as of August 2024.

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Figure Legend

Figure 1: Classification changes of 35 gene-disease pairs recurated from 2020–2024. *AD neonatal diabetes, congenital sensorineural hearing loss and congenital cataracts, originally classified as Strong, was lumped with AD Wolfram-like syndrome which was classified as Definitive for *WFS1* in 2018

Table. 1: Summary of 35 gene-disease pairs recurated from 2020–2024.

Gene	HGNC ID	Inheritance	Disease	Initial Classification (SOP, year)	New evidence (genetic/functional)	Recuration Classification (SOP, year)
BDP1	13652	AR	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2017)	No new evidence	Limited (v8, 2021)
CCDC50	18111	AD	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2017)	Genetic	Limited (v10, 2024)
CD164	1632	AD	autosomal dominant nonsyndromic deafness (MONDO:0019587)	Limited (v6, 2018)	Genetic	Limited (v10, 2024)
CDC14A	1718	AR	hearing impairment and infertile male syndrome (MONDO:0100069)	Strong (v5, 2018)	No new evidence	Strong (v9, 2023)
CEACAM16	31948	AD	nonsyndromic genetic deafness (MONDO:0019497)	Moderate (v5, 2018)	Genetic	Moderate (v9, 2022)
CISD2	24212	AR	Wolfram syndrome (MONDO:0018105)	Strong (v6, 2018)	Both	Definitive (v9, 2023)
COL4A6	2208	X-linked	deafness, X-linked 6 (MONDO:0010484)	Limited (v6, 2018)	No new evidence	Limited (v8, 2022)
COL9A3	2219	AR	Stickler syndrome (MONDO:0019354)	Moderate (v7, 2019)	Genetic	Definitive (v9, 2022)
CRYM	2418	AD	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v5, 2017)	Genetic	Limited (v8, 2021)
DIABLO	21528	AD	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2017)	Genetic	Limited (v8, 2021)
EDN3	3178	AR	Waardenburg syndrome type 4B (MONDO:0013201)	Moderate (v6, 2018)	Genetic	Moderate (v9, 2023)
EDNRB	3180	AR	Waardenburg syndrome type 4A (MONDO:0010192)	Moderate (v5, 2018)	Genetic	Moderate (v9, 2023)
ELMOD3	26158	AR	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2017)	Functional	Limited (v8, 2021)
FOXI1	3815	AR	hearing loss (MONDO:0005365)	Limited (v6, 2018)	No new evidence	Limited (v8, 2022)
GJA1	4274	AD	nonsyndromic genetic deafness (MONDO:0019497)	Disputed (v6, 2018)	No new evidence	Disputed (v8, 2022)
GJB3	4285	AD	erythrokeratodermia variabilis (MONDO:0017851)	Strong (v6, 2018)	Genetic	Definitive (v9, 2023)
HGF	4893	AR	nonsyndromic genetic deafness (MONDO:0019497)	Moderate (v5, 2018)	No new evidence	Moderate (v10, 2024)
LARS2	17095	AR	Perrault syndrome (MONDO:0017312)	Strong (v5, 2018)	Genetic	Definitive (v9, 2023)
MET	7029	AR	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v5, 2017)	Both	Limited (v8, 2021)
MSRB3	27375	AR	nonsyndromic genetic deafness (MONDO:0019497)	Moderate (v5, 2017)	Genetic	Definitive (v8, 2021)
MYO3A	7601	AR	nonsyndromic genetic deafness (MONDO:0019497)	Strong (v6, 2017)	Genetic	Definitive (v9, 2023)
NARS2	26274	AR	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2017)	No new evidence	Limited (v8, 2021)
P2RX2	15459	AD	nonsyndromic genetic deafness (MONDO:0019497)	Moderate (v5, 2018)	Both	Moderate (v9, 2022)
ROR1	10256	AR	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v5, 2018)	Functional	Limited (v9, 2022)
SERPINB6	8950	AR	nonsyndromic genetic deafness (MONDO:0019497)	Moderate (v6, 2018)	Genetic	Moderate (v9, 2022)
SLC17A8	20151	AD	nonsyndromic genetic deafness (MONDO:0019497)	Strong (v6, 2018)	Genetic	Definitive (v9, 2023)
SLC26A5	9359	AR	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v5, 2017)	No new evidence	Limited (v8, 2022)
SLC44A4	13941	AD	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2018)	No new evidence	Limited (v9, 2022)
SYNE4	26703	AR	nonsyndromic genetic deafness (MONDO:0019497)	Moderate (v5, 2017)	Genetic	Moderate (v7, 2020)
TBC1D24	29203	AD	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2018)	Both	Limited (v10, 2024)
TJP2	11828	AD	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2017)	No new evidence	Limited (v8, 2022)
TMEM132E	26991	AR	autosomal recessive nonsyndromic deafness (MONDO:0019588)	Limited (v5, 2017)	Genetic	Limited (v8, 2022)
TMTC2	25440	AD	nonsyndromic genetic deafness (MONDO:0019497)	Disputed (v5, 2017)	No new evidence	Disputed (v9, 2022)
TNC	5318	AD	nonsyndromic genetic deafness (MONDO:0019497)	Limited (v6, 2018)	Genetic	Limited (v9, 2022)
WFS1	12762	AD	neonatal diabetes, congenital sensorineural hearing loss and congenital cataracts (MONDO:0100072)	Strong (v6, 2018)	Genetic	Lumped w/ AD WFS-like (Definitive) (v9, 2023)

Gene-disease Validity Recuration Results of 35 HL Genes