Improving Genetic Diagnosis Yield for Hearing Loss Through Reclassification of ClinGen Limited Evidence Genes

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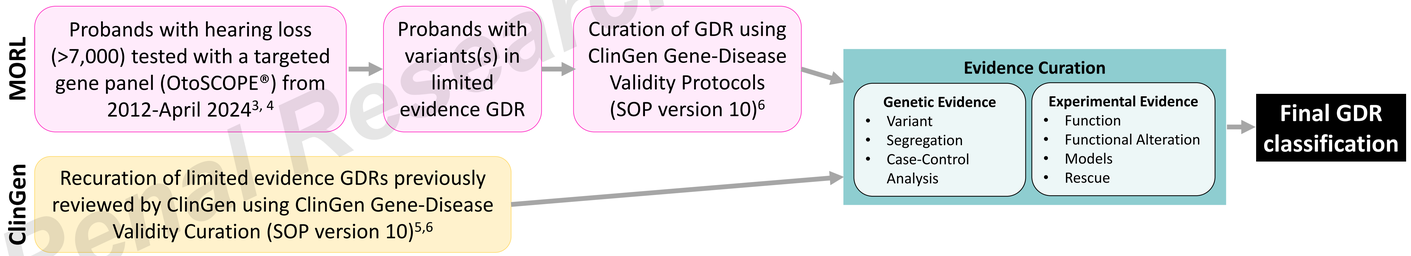
Introduction

- Accurate genetic diagnosis of hearing loss relies on robust evidence supporting gene-disease relationships (GDRs) and disease-focused classification of genetic variants.
- The Clinical Genome Resource (ClinGen) has developed a semiquantitative framework to evaluate GDRs categorizing the strength of the GDR as: definitive, strong, moderate, limited, disputed, or refuted.
- Limited evidence GDRs are those where there is plausible evidence to support a GDR, but additional data are needed to confirm the relationship[1].
- The American College of Medical Genetics and Genomics (ACMG) recommends excluding limited evidence GDRs from clinical genetic testing panels[2].

Here, we identified emerging evidence for GDRs classified as limited evidence by the ClinGen Hearing Loss Gene Curation Expert Panel, resulting in reclassification of three GDRs for hearing loss.

Methods



Results

HARS2: Perrault Syndrome Type 2

Clinical features of Perrault syndrome type 2

- *Hearing loss:* variable onset and severity, most commonly congenital, moderately severe-to-profound sensorineural hearing loss (SNHL), progression has been reported
- Ovarian dysfunction in females ranging from primary ovarian insufficiency to ovarian dysgenesis

Table 1: MORL probands with Perrault syndrome type 2

ID	Age	Sex	Ancestry/ Ethnicity	Hearing Loss	Familial testing	Variant	Zygosity	Max MAF (%) pop	REVEL	Reference
1	10 y	M	Asian	congenital bilateral profound SNHL	In *trans*	c.448C>T, p.R150C	het	0.01 AFR	0.62	PMID: 31449985
						c.1504C>T, p.Arg502*	het	–	NA	–
2	6 m	FM	European/ non-Hispanic	congenital bilateral	–	c.448C>T, p.R150C	het	0.01 AFR	0.62	PMID: 31449985
						c.1091A>G, p.Y364C	het	0.004 AFR	0.9	–
3	13 y	FM	Hispanic	mild-to-moderate mid-frequency SNHL diagnosed at 12 y	–	c.697C>T, p.R233C	hom	0.006 AFR	0.31	PMID: 31486067; ClinVar: SCV000271838.2
4	3 y	FM	Hispanic	bilateral profound SNHL diagnosed at 2 y	–	c.697C>T, p.R233C	hom	0.006 AFR	0.31	PMID: 31486067; ClinVar: SCV000271838.2

Abbreviations: Max MAF: maximum minor allele frequency, pop: Genome Aggregation Database population, REVEL: rare exome variant ensemble learner, y: years, m: months, M: male, FM: female, het: heterozygous, hom: homozygous, AFR: African/African American, NA: not applicable. *HARS2* variants annotated on NM_012208.

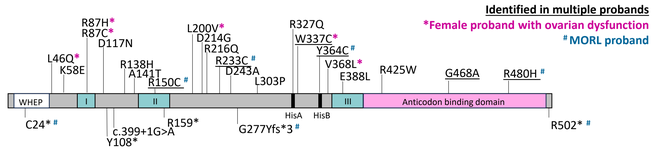


Figure 1: Causative *HARS2* variants reported in literature, ClinVar, and MORL cohort.

Domains/regions: WHEP: 3-43, Motif I: 80-97, Motif II: 147-181, HisA: 327-332, HisB: 361-365, Motif III: 378-393, Class II aminoacyl tRNA synthetase like core domain: 80-393, Anticodon binding domain AA: 406-501

Table 2: GDR curation for *HARS2*

	Evidence Type	ClinGen	Literature	MORL	Total points
Genetic Evidence	Variant	1	5	0.5	8.5
	Segregation	2	–	–	
Experimental Evidence	Function	0.5	–	–	3.5
	Models	1	2	–	
	Total points	4.5	7	0.5	TOTAL POINTS: 12

Reclassification of GDR to STRONG for *HARS2* and Perrault syndrome type 2 → **Genetic diagnosis for 4 probands**

EDNRB: Autosomal Dominant Waardenburg Syndrome type 4A

Clinical features of Waardenburg syndrome type 4A

- *Hearing loss:* variable onset and severity, most commonly congenital SNHL
- *Pigmentary changes:* variable expressivity of features including white forelock, early graying, heterochromia, hypoplastic/brilliant blue irises, skin hypopigmentation
- Hirschsprung's disease in some

Table 3: MORL probands with Waardenburg syndrome type 4A

ID	Age	Sex	Ancestry/ Ethnicity	Hearing Loss	Physical Exam	Family History	Familial Testing	Variant	Zygosity	Max MAF (%) pop	REVEL	Reference
5	10 y	M	African, African American	congenital moderate-to-severe SNHL	blue eyes, pigmentary retinal dystrophy	Mother: skin hypopigmentation MU: blue eyes, constipation MGF: blue eyes	–	c.872G>C, p.R291P	het	0.0008 NFE	0.56	–
6	1 y	FM	African, African American	congenital asymmetric SNHL	narrow ear canals, ambiguous genitalia	Neg	–	c.46delC, p.R16Gfs*60	het	0.014 AFR *unseen in other populations	NA	–
7	6 m	M	African, African American	congenital severe-to-profound bilateral SNHL	blue eyes, skin hypopigmentation, white patch of hair	Mother: unilateral HL, blue eyes	Mother: positive for variant	c.1114T>G, p.F372V	het	–	0.93	–
8	2 y	M	–	congenital profound bilateral SNHL	normal	Mother, MGF: heterochromia distant maternal relatives: HL, Hirschsprung's	–	c.1098G>A, p.W366*	het	–	NA	–
9	5 m	M	European/ non-Hispanic	congenital unilateral severe-to-profound SNHL	heterochromia	S: telecanthus F, PGM, PGA: Hirschsprung's	S, F: positive for variant	c.871C>T, p.R291*	het	0.0008 NFE	NA	PMID: 11891690; ClinVar: SCV00124 8436.24, SCV00078 1313.1

Abbreviations: Max MAF: maximum minor allele frequency, pop: Genome Aggregation Database population, REVEL: rare exome variant ensemble learner, y: years, m: months, M: male, FM: female, MU: maternal uncle, MGF: maternal grandfather, S: sister, F: father, PGM: paternal grandmother, PGA: paternal great aunt, het: heterozygous, hom: homozygous, AFR: African/African American, NFE: European (non-Finnish), NA: not applicable. *EDNRB* variants annotated on NM_001201397.1.

Table 4: GDR curation for *EDNRB*

	Evidence Type	ClinGen	MORL	Total points
Genetic Evidence	Variant	2.5	4.5	7
	Total points	2.5	4.5	TOTAL POINTS: 7

Reclassification of GDR to MODERATE for *EDNRB* and autosomal dominant Waardenburg syndrome type 4A → **Genetic diagnosis for 5 probands**

SLC26A5: Autosomal Recessive Non-Syndromic Hearing Loss

Clinical features of *SLC26A5*-related non-syndromic hearing loss

- Congenital and bilateral with moderate, severe, and profound severity reported

Table 5: MORL probands with SLC26A5-related non-syndromic HL

ID	Age	Sex	Ancestry/ Ethnicity	Hearing Loss	Familial Testing	Variant	Zygosity	Max MAF (%) pop	REVEL	Reference
10	31 y	M	European	Congenital bilateral severe-to-profound SNHL	In *trans*	c.154T>G, p.C52G	het	0.0005 NFE	0.94	–
						deletion exons 9-14	het	–	NA	–
11	9 m	FM	European	Congenital bilateral moderate-to-moderately-severe SNHL	–	c.2036G>A, p.C679Y	het	0.005 EAS	0.79	–
						duplication exons 3-14	het	–	NA	–

Abbreviations: Max MAF: maximum minor allele frequency, pop: Genome Aggregation Database population, REVEL: rare exome variant ensemble learner, y: years, m: months, M: male, FM: female, het: heterozygous, hom: homozygous, NFE: European (non-Finnish), EAS: East Asian, NA: not applicable. *SLC26A5* variants annotated on NM_198999.3.

Table 6: GDR curation for *SLC26A5*

	Evidence Type	ClinGen	MORL	Total points
Genetic Evidence	Variant	2	1.5	3.5
Experimental Evidence	Function	2	–	6
	Models	4	–	
	Total points	8	1.5	TOTAL POINTS: 9.5

Reclassification of GDR to MODERATE for *SLC26A5* and non-syndromic hearing loss → **Genetic diagnosis for 2 probands**

Conclusions

Review of a large cohort of persons with hearing loss for variant(s) in limited evidence GDRs led to reclassification of three GDRs:

- *HARS2* and autosomal recessive Perrault syndrome type 2
- *EDNRB* and autosomal dominant Waardenburg syndrome type 4A
- *SLC26A5* and autosomal recessive non-syndromic hearing loss

Our findings demonstrate the power of reviewing variants in limited evidence genes to strengthen GDRs and improve genetic diagnostic yield for hearing loss.

- Reclassifications resulted in a genetic diagnosis for 11 probands

These results are a call to action for testing laboratories to participate in data sharing for GDRs.

References and Acknowledgements

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