Integrating Phenotypic and Genotypic Data to Enhance Diagnosis and Clinical Care of Persons with Hearing Loss

Amanda O. Taylor[1], Carla J. Nishimura[1], Kathy L. Frees[1], Diana L. Kolbe[1], Kevin T. Booth[1,2], Robert J. Marini[1], Donghong Wang[1], Amanda M. Schaefer[1], Paige L. Harlan[1], Amy E. Weaver, Jori E. Hendon[1], Hela Azaiez[1], Richard J.H. Smith[1]

[1] Molecular Otolaryngology and Renal Research Laboratories, Department of Otolaryngology—Head and Neck Surgery, University of Iowa Hospitals and Clinics, Iowa City, Iowa, USA

[2] Department of Neurobiology – Harvard Medical School, Boston, Massachusetts, USA

Introduction

- Deafness is the most common sensory deficit and affects 1 in 500 newborns.
- Over 150 genes have been associated with hearing loss (HL) and associated syndromes.
- This heterogeneity makes next-generation sequencing (NGS) the recommended test in the evaluation of deaf/hard-of-hearing persons.
- Allelic, genetic and phenotypic heterogeneity can make interpretation of variants found via NGS difficult to assess.

Here we present six representative cases in which phenotypic data were used to facilitate the interpretation of genetic findings in patients with deafness.

Methods

- A custom targeted genomic enrichment and massively parallel sequencing panel (TGE+MPS), OtoSCOPE®, was used to screen 152 hearing loss-associated genes and multiple common syndromic forms of HL.
  - Methodology includes Agilent Sure Design, Illumina HiSeq or NextSeq sequencing and a custom bioinformatics pipeline.
  - Single nucleotide variant (SNV) filtering: QD≥5; Qvar≥50; MAF<2%; non-synonymous, indels and splice-site variants
- Copy number variations (CNVs) varying in size from single exon to whole gene were identified using a previously published tool that normalizes read-depth data by sample batch and compares average read-depth ratios using a sliding-window approach followed by manual curation.
- Results were discussed at a multidisciplinary meeting with physicians, research scientists, geneticists, bioinformaticians, and genetic counselors in the context of the patient's clinical information including:
  - Clinical history, physical exam, family history, audiometric data (audiograms, progression, severity and laterality) and age at diagnosis.

Cases

Case 1

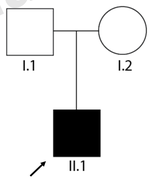


Table 1: Causative SNV identified via TGE+MPS for the proband; Abbreviations: AA: amino acid, MAF: minor allele frequency, Pop: population, NFE: non-Finnish European; ASJ: Ashkenazi Jewish, SAS: South Asian, Het: heterozygous, Hom: homozygous; GERP: GERP++ RS, PP2: Polyphen2 HDIV, DVD: Deafness Variation Database, ND: no data, C: conserved, NC: not conserved, D: damaging, PD: possibly damaging, P: polymorphism, N: Neutral; Path: pathogenic, Unkn Sig: variant of unknown significance

Gene	Nuc. Change	AA Change	Zygosity	MAF (%) Max GnomAD	MAF (%) Max Pop	GERP	PhyloP	PP2	SIFT	Mutation Taster	LRT	CADD	DVD
PTPRQ	c.2133G>T	p.G711*	het	ND	ND	C	C	ND	ND	D	ND	44	ND

ACMG Criteria: pathogenic (PVS1, PM2, PP3, PP4)

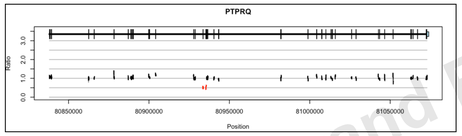


Figure 1: Heterozygous deletion of exons 17 and 18 in *PTPRQ* identified in the proband

Clinical Information:
- 4-year-old Hispanic Caucasian male
- Congenital mild-moderate hearing loss
- Clinical history: delayed motor milestones, walked at 15 months-of-age
- Family history: negative

Diagnosis:
Diagnosis of DFNB84 and vestibular dysfunction due to two pathogenic variants in *PTPRQ:*
- Pathogenic nonsense variant
- Pathogenic 2-exon deletion

Significance:
- Genetic data refines the diagnosis and alters the prognosis and course of clinical care away from Usher syndrome when patient presented with delayed motor milestones

Case 2

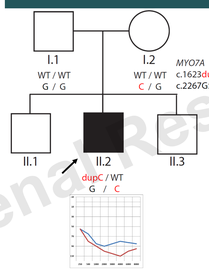


Figure 2: Pedigree and audiometric data. Segregation analysis of the *MYO7A* variants identified in the proband reveals the small duplication to be *de novo* and the missense to be inherited from I.2.

Table 2: Causative SNVs identified in the proband by TGE+MPS.

Gene	Nuc. Change	AA Change	Zygosity	MAF (%) Max GnomAD	MAF (%) Max Pop	GERP	PhyloP	PP2	SIFT	Mutation Taster	LRT	CADD	DVD
MYO7A	c.1623dupC	p.K542Qfs*5	het	0.0033%	SAS	ND	ND	ND	ND	ND	ND	34	path
MYO7A	c.2267G>C	p.R756P	het	ND	ND	C	C	D	D	D	D	34	ND

ACMG Criteria: Variant 1 - pathogenic (PVS1, PM2, PS2, PP3, PP4); Variant 2 – likely pathogenic (PM2, PM5, PP3, PP4)

Clinical Information:
- 5-year-old Asian male
- Moderate-profound SNHL
- Clinical history: delayed motor milestones – walking at 18 months and reported to have continued balance problems
- Family history: negative

Diagnosis:
Diagnosis of USH1B due to two variants in *MYO7A:*
- Pathogenic *de novo* truncating variant
- Likely pathogenic missense variant at same amino acid residue previously reported for USH1B

Significance:
- Delayed motor milestones alongside expert curation directed diagnosis of USH1B versus DFNB12
- Segregation analysis reveals a *de novo* variant in this family which impacts family planning and genetic counseling

Case 3

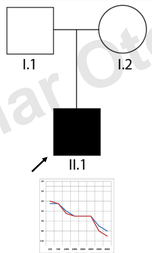


Table 3: Causative SNVs identified in individual II.1 by TGE+MPS

Gene	Nuc. Change	AA Change	Zygosity	MAF (%) Max GnomAD	MAF (%) Max Pop	GERP	PhyloP	PP2	SIFT	Mutation Taster	LRT	CADD	DVD
WFS1	c.1619G>A	p.W540*	het	0.0088%	NFE	C	C	ND	ND	D	D	38	path
WFS1	c.1630T>C	p.S544P	het	0.0008%	NFE	NC	C	PD	D	P	N	17.02	ND

ACMG Criteria: Variant 1 - pathogenic (PVS1, PM2, PP3, PP4, PP5); Variant 2 – likely pathogenic (PM2, PM3, PM5, PP3, PP4)

Figure 3: Audiogram data for the proband

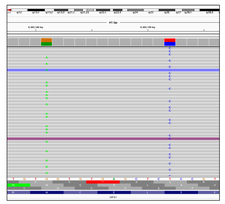


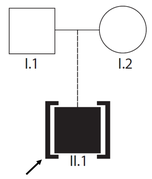
Figure 4: IGV view showing the two *WFS1* variants ***in trans***

Clinical Information:
- 10-year-old male
- Mild-to-profound HL
- Clinical history: Type 1 Diabetes diagnosed at 4 years of age
- Family history: negative

Diagnosis:
Diagnosis of Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness syndrome (DIDMOAD) due to two variants in *WFS1*:
- Pathogenic nonsense variant
- Likely pathogenic missense variant

Significance:
- Type 1 Diabetes in the proband informed the diagnosis of two *WFS1* variants significant for DIDMOAD
- This genetic diagnosis will change the course of the patient's clinical care

Case 4



Clinical Information:
- 4-year-old Fijian-Indian male
- Congenital, profound, bilateral SNHL
- Clinical history: sapphire-blue eyes
- Family history: unknown – patient is adopted
- Medical imaging: CT scan - inner ear dysplasia and absent posterior semicircular canals

Table 4: Causative splice variant identified in proband

Gene	Nuc. Change	AA Change	Zygosity	MAF (%) Max GnomAD	MAF (%) Max Pop	GERP	PhyloP	PP2	SIFT	Mutation Taster	LRT	CADD	DVD
SOX10	c.428+2T>G	- - -	het	ND	ND	C	C	ND	ND	D	ND	23.7	path

ACMG Criteria: pathogenic (PVS1, PM2, PP3, PP4, PP5)

Diagnosis:
Diagnosis of Waardenburg Syndrome type 2E/4C due to a variant in *SOX10*:
- Pathogenic canonical splice-site variant in *SOX10*

Significance:
- Strong phenotypic data facilitates the genetic diagnosis of this patient and the pathogenicity classification

Case 5

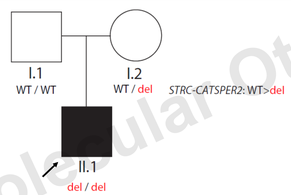


Figure 5: Segregation analysis for the homozygous deletion of *STRC-CATSPER2* identified in individual II.1

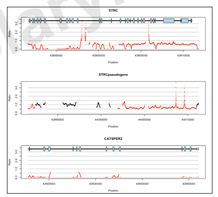


Figure 7: A homozygous deletion in the *STRC-CATSPER2* genomic region identified via TGE+MPS in the proband.

Clinical Information:
- 1-year-old Caucasian male
- Congenital SNHL
- Clinical history: hypotonia
- Family history: negative

Diagnosis:
Diagnosis of Deafness-Infertility syndrome:
- Contiguous gene deletion of *STRC-CATSPER2*

Significance:
- Deafness-Infertility syndrome is significant for reproductive care in males
- Segregation analysis reveals a *de novo* variant in this family, which will be significant for genetic counseling and family planning for Individuals I.1 and I.2

Case 6

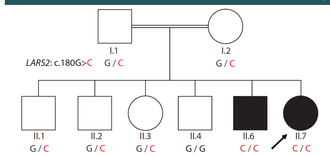


Figure 6: Segregation analysis of the *LARS2* variant indicating segregation in the extended family

Clinical Information:
- 7-year-old Ashkenazi Jewish female
- Early-childhood moderate-severe upsloping SNHL
- Family history: brother with HL
- Previous genetic testing: negative

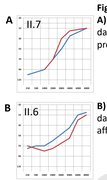


Figure 7:
A) Audiometric data for the proband
B) Audiometric data for the affected sibling

Table 5: Diagnostic SNV identified in the proband

Gene	Nuc. Change	AA Change	Zygosity	MAF (%) Max GnomAD	MAF (%) Max Pop	MAF (%) Max Outbred GnomAD	MAF (%) Max Outbred Pop	GERP	PhyloP	PP2	SIFT	Mutation Taster	LRT	CADD	DVD
LARS2	c.180G>C	p.E60D	hom	0.24%	ASJ	0.0008%	NFE	C	C	D	D	D	D	28.6	unkn sig

ACMG Criteria: variant of unknown significance (PM2, PP1, PP3, PP4)

Diagnosis:
Perrault syndrome due a variant in *LARS2*:
- A pathogenic missense variant

Significance:
- *LARS2* causes Perrault syndrome which manifests in affected males and females differently. Both sexes have progressive HL but females also display premature ovarian failure which will affect their reproductive health.
- A possible founder mutation significant for Perrault syndrome could impact carrier screening in the Ashkenazi Jewish population when presenting with an upsloping audiogram.

Conclusions

These six cases showcase that:
- Genetic results can refine a patient's diagnosis and alter clinical care and prognosis
- Phenotypic data and segregation analysis improve the accuracy of genetic variant classification

Therefore:
- Establishing a genetic diagnosis supported by phenotypic data improves and informs patients' clinical care

And we recommend:
- All persons with hearing loss who undergo genetic testing provide:
  - Clinical information
  - Family history
  - Audiometric data
  - The genetic data be comprehensive and include:
    - Single nucleotide variant analysis
    - Copy number variant analysis
  - All data be interpreted by a multidisciplinary team of experts (comprising geneticists, clinicians, bioinformaticians and genetic counselors) who use phenotypic data to guide the interpretation of genetic findings.

References

- Azaiez, H., et al (2018). "Genomic Landscape and Mutational Signatures of Deafness-Associated Genes" AM J Hum Genet. 103(4):484-497. PMID: 30245029
- Sloan-Heggen CM. (2016). "Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss" Hum Genet. 135(4):441-450. PMID: 26969326.
- Zhang, Y., et al. (2007). "Sensorineural deafness and male infertility: a contiguous gene deletion syndrome" J Med Genet. 44(4):233-40. PMID: 17098888

Acknowledgements

This work was supported in part by NIH DC012049 to RJHS.
We are grateful to the healthcare providers and, most of all, the patients who have allowed us to participate in their care.