Dual Diagnoses of Genetic Hearing Loss Identified on Multigene Panels: Considerations for Clinical Care and Genetic Counseling

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Introduction

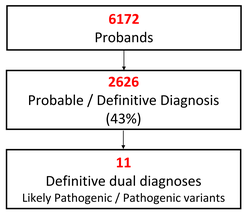
- Genetic testing is a vital component of care for deaf and hard-of-hearing persons.
- The American College of Medical Genetics and Genomics (ACMG) recommends a multigene hearing loss (HL) panel for patients with non-syndromic HL.[1]
- Multigene HL panel testing may identify dual genetic diagnoses, presenting a challenge for genetic counseling and clinical care.
- There is little data about the frequency and subsequent challenges in genetic counseling for dual diagnoses of HL.

Here, we present a series of cases with dual genetic diagnoses identified on a multigene HL panel to highlight the complexities of genetic counseling and clinical care.

Methods

- **Subjects:** Ethnically diverse cohort with hearing loss ascertained from 2012 through June 2022.
- **Genetic testing:** Targeted genomic enrichment and massively parallel sequencing to screen all non-syndromic HL-associated genes and common syndromic genes (OtoSCOPE).
- **Bioinformatic analysis:** Customized Galaxy pipeline and analysis for single nucleotide variants and analysis of normalized read-depth data by sample batch compared to average read-depth for copy number variation (CNV) identification.
- **Genetic results:** Genetic findings were discussed at a multidisciplinary meeting with physicians, geneticists, bioinformaticians, and genetic counselors in the context of the patient's clinical data and history.
- **Variant classification:** All variants were classified using ACMG/AMP Hearing Loss Specific classification guidelines.[4]

Cohort Identification



Dual diagnoses seen in ~1 in 250 probands

Figure 1. MORL dual diagnosis cohort identification: Of ~2600 probands with a probable genetic diagnosis, 11 probands (0.4%) were identified with dual genetic diagnoses, defined as Likely Pathogenic or Pathogenic variant(s) in 2 genes.

Dual Diagnoses Cohort

Table 1. Dual diagnoses case details.

Proband	Age at testing	HL Phenotype	Physical exam	Family history	Diagnosis 1			Diagnosis 2		
					Gene	Diagnosis	Inheritance	Gene	Diagnosis	Inheritance
1	7 m	Congenital moderate SNHL	Right ptosis	Negative; parental consanguinity	*GJB2*	DFNB1	AR	*OTOA*	DFNB22	AR
2	5 m	Congenital moderate SNHL	Normal	Negative	*GJB2*	DFNB1	AR	*OTOA*	DFNB22	AR
3	1 y	Not provided	Not provided	Not provided	*GJB2*	DFNB1	AR	*TECTA*	DFNA12	AD
4	7 y	Congenital moderate-to-severe SNHL	Normal	Negative	*GJB2*	DFNB1	AR	*MT-TL1*	MELAS / MIDD	M
5	2 m	Congenital profound SNHL	Normal	2 paternal uncles: unilateral HL	*GJB2*	DFNB1	AR	*USH2A*	Usher type 2A / non-syndromic retinitis pigmentosa	AR
6	5 y	Congenital profound SNHL	Normal	Younger brother: profound HL	*GJB2*	DFNB1	AR	*COL11A2*	non-ocular Stickler type 3	AD
7	3 y	Congenital moderate SNHL	Normal	Negative	*GJB2*	DFNA3A	AD	*COL4A3*	familial hematuria	AD
8	15 y	Early childhood onset SNHL	Normal	Mother, two maternal half siblings: bilateral HL, maternal uncle: unilateral HL	*TBX1*	22q11.2 duplication syndrome	AD	*MT-RNR1*	aminoglycoside- induced HL	M
9	1 m	Congenital mild-to-moderate SNHL	Normal	Negative	*TBX1*	22q11.2 duplication syndrome	AD	*GATA3*	Hypoparathyroidism, Deafness, Renal disease (HDR)	AD
10	6 y	Early childhood onset SNHL	Normal	Negative	*TBX1*	22q11.2 deletion syndrome	AD	*MYO7A*	DFNA11	AD
11	8 y	Early childhood onset mild-to-moderate SNHL	Normal	Negative	*KCNQ4*	DFNA2A	AD	*EYA1*	Branchiootorenal (BOR) syndrome	AD

Abbreviations: SNHL: sensorineural hearing loss, AD: autosomal dominant, AR: autosomal recessive, M: mitochondrial, MELAS: Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes, MIDD: Maternally Inherited Diabetes and Deafness

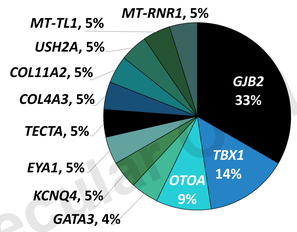


Figure 2. Causative genes in dual diagnoses cohort. A total of 12 genes were identified as causative in this cohort. *GJB2*, *TBX1*, and *OTOA* were the most frequent, comprising ~55% of diagnoses.

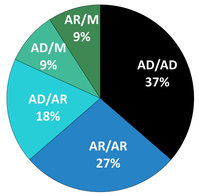


Figure 3. Inheritance pattern combinations identified. A variety of inheritance pattern combinations were seen with 2 AD or 2 AR being most common. (AD: autosomal dominant, AR: autosomal recessive, M: mitochondrial)

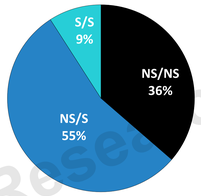


Figure 4. Syndromic versus non-syndromic diagnoses. Around 2/3 of probands had at least 1 syndromic diagnosis. (S: syndromic, NS: non-syndromic)

Illustrative Cases

Proband 5

Clinical Information:

- 2-month-old white male
- Congenital bilateral severe-to-profound SNHL
- Family history: negative

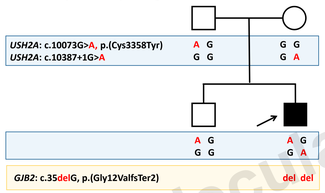


Figure 5: Pedigree showing segregation of *USH2A* variants.

Table 2: Causative variants identified

Gene	Nuc. Change	AA Change	Max MAF gnomAD	ACMG Classification	ACMG Criteria Applied
USH2A	c.10073G>A	p.(Cys3358Tyr)	0.076% (NFE)	Pathogenic	BS1_Sup, PM3_VS, PP4, PP3
USH2A	c.10387+1G>A	NA	ND	Pathogenic	PVS1, PM2, PM3
GJB2	c.35delG	p.(Gly12ValfsTer2)	0.84% (NFE)	Pathogenic	PVS1, PM3_VS, PS4, BA1

Abbreviations: Nuc: nucleotide, AA: amino acid, MAF: minor allele frequency, NFE: non-Finnish European, NA: Not applicable, BS1_Sup: BS1_Supporting, PM3_VS: PM3_VeryStrong

Diagnoses:

1. Autosomal recessive non-syndromic HL (DFNB1)
2. Usher syndrome type 2A or autosomal recessive non-syndromic retinitis pigmentosa (ARRP)

DFNB1-related HL phenotype masks *USH2A*-related phenotype

Impact to genetic counseling and clinical care:

1. Proband referred to ophthalmology
2. DFNB1-related HL masks *USH2A*-related HL phenotype
3. Prompts genetic testing for normal hearing brother
   - Biallelic loss of function variants are more often seen in Usher type 2A and biallelic missense in autosomal recessive non-syndromic retinitis pigmentosa (RP).
   - Identified missense variant enriched in non-syndromic RP. [5]
   - Brother could have inherited the second *USH2A* variant and developed non-syndromic RP despite normal hearing.

Proband 9

Clinical Information:

- 1-month-old White, Asian female
- Congenital mild-to-moderate bilateral SNHL
- Family history: adopted embryo, no HL reported in biological parents
- Physical exam: normal

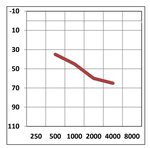


Figure 5: Audiogram of proband.

Diagnoses:

1) 22q11.2 duplication syndrome
2) Hypoparathyroidism, deafness, renal disease (HDR) syndrome

Table 3: Causative variants identified

Gene	Nuc. Change	AA Change	Max MAF gnomAD	ACMG Classification	ACMG Criteria Applied
TBX1	whole gene duplication	NA	ND	Pathogenic	PVS1, PS4_S
GATA3	c.708del	p.(Ser237AlafsTer29)	ND	Pathogenic	PVS1, PM2, PS4_Mod

Abbreviations: AA: amino acid, MAF: minor allele frequency, ND: no data, PS4_S: PS4_Strong, PS4_Mod: PS4_Moderate

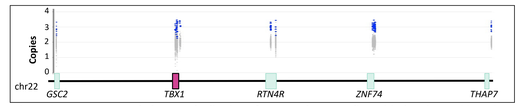


Figure 6: Heterozygous whole gene duplication of *TBX1* and surrounding critical region at 22q11.2. Dots represent normalized read-depth data by sample batch compared to average read-depth, blue : proband, grey: unrelated probands in testing batch. To determine extent of duplication, referring healthcare provider ordered a chromosome microarray from an outside lab which confirmed the diagnosis of 22q11.2 duplication syndrome due to a pathogenic 2.5 Mb duplication: arr[GRCh37] 22q11.21(18915102_21466669)X3.

Impact to genetic counseling and clinical care:

1. Proband referred to endocrinology, nephrology, and developmental and educational support organizations
2. Education on variability of both diagnoses [6,7]
3. Blending of phenotypes and challenge of providing prognostic information on HL and other features.

22q11.2 duplication syndrome phenotype blends with HDR syndrome phenotype

Conclusions

1. **The possibility of dual genetic diagnoses cannot be overlooked with multigene HL panel testing.**

   Dual diagnoses were seen in ~1 in 250 probands.

2. **Multigene panel testing is recommended over single gene testing for *GJB2*.**

   ~1 in 100 probands diagnosed with DFNB1-related HL had a second genetic diagnosis identified.

3. **Pre-test counseling should include discussion of non-syndromic mimics.**

   ~2/3 of probands with dual diagnoses were identified to have a genetic syndrome, despite normal physical exams.

Our data highlight the importance of considering dual genetic diagnoses and the complexity of genetic counseling and clinical care for these families.

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Acknowledgements

This work was supported in part by NIH DC012049 to RJHS. We are grateful to the healthcare providers, patients, and families who have allowed us to participate in their care. Questions can be directed to Amanda Schaefer, MS, LGC (Amanda-Schaefer-1@uiowa.edu).