Defining the Genetic Landscape of *OTOA*-related Hearing Loss

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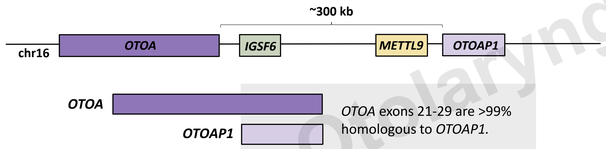
Introduction

- *OTOA* is responsible for autosomal recessive non-syndromic hearing loss at the DFNB22 locus.
- Copy number variants (CNVs) are the most frequently reported variant in *OTOA* and reflect non-allelic recombination due to a nearby highly homologous pseudogene (*OTOAP1*).

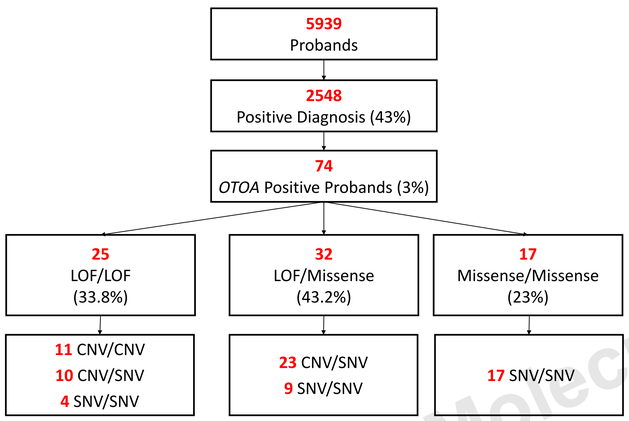


- Here, we present the largest patient cohort exploring the mutational landscape of *OTOA* mutations and hearing loss phenotypes.

Methods

- **Subjects:** ethnically diverse cohort with hearing loss ascertained from 2012 through 2021.
- **Genetic testing:** targeted gene panel (OtoSCOPE®) was used to screen all non-syndromic hearing loss-associated genes and multiple common syndromic forms of hearing loss.
  - **Genomic enrichment and massively parallel sequencing and bioinformatic analysis**: Agilent SureSelect Design, Illumina HiSeq or NextSeq sequencing and a customized Galaxy pipeline used for bioinformatics analysis.
  - **Single nucleotide variant (SNV) filtering**: QD≥5; Qvar≥50; MAF<2%
  - **Copy number variation (CNV) identification**: analysis of normalized read-depth data by sample batch compared to average read-depth followed by manual curation.
  - **Manual review of sequencing reads:** performed using Integrated Genomics Viewer (IGV).
- Genetic results were discussed at a multidisciplinary meeting with physicians, geneticists, bioinformaticians, and genetic counselors in the context of the patient's clinical data and history.

Genetic Testing Results



Causative alleles in **14 of 74 probands** were identified following manual review of sequencing reads in highly homologous regions between gene and pseudogene

23% increase in diagnostic yield

Figure 1. MORL *OTOA* cohort identification and genotypes. LOF variants include: stop gain, splice, frameshift indel variants, CNVs, and gene-to-pseudogene conversions.

Mutation Prevalence by Type

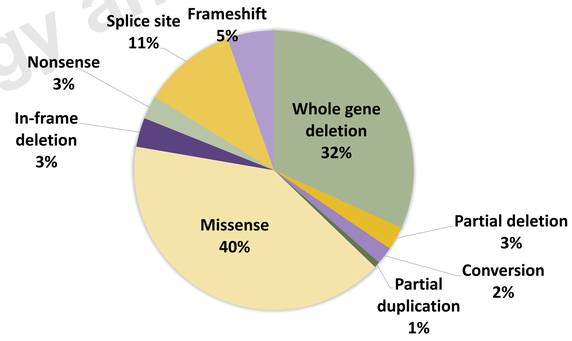


Figure 2. Prevalence of causative alleles in MORL cohort. A whole gene deletion was the single most frequently detected variant: 36 probands (48.6%), representing 32% of causative alleles (47/148). Missense variants were responsible for 40% of causative alleles.

Audiometric Analysis

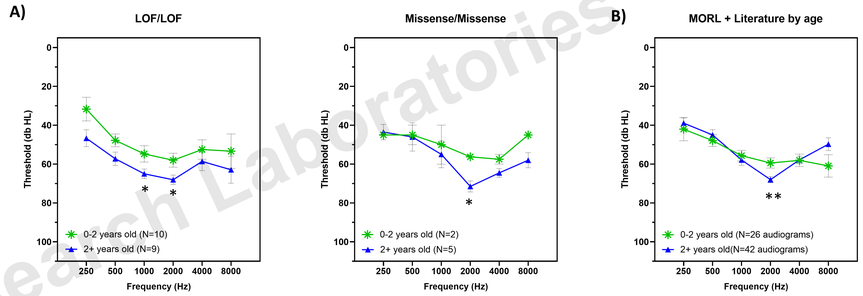


Figure 3. Pure-tone average of *OTOA*-related hearing loss (MORL cohort + published literature). A) Probands were categorized by *OTOA* genotype and age. Data represent mean ± SEM of the average hearing loss threshold. There was a statistically significant differences in thresholds between 0-2 years old group and 2+ years old group for LOF/LOF at 1000 and 2000 Hz (p= 0.048 and p= 0.03 respectively), and Missense/Missense at 2000 Hz (p=0.02). **B)** Analysis of all available audiograms for OTOA by age. A statistically significance difference in threshold was identified at 2000 Hz (p=0.003). Groups were compared using a test. *p<0.05,** p < 0.005.

Mutational Landscape of *OTOA*

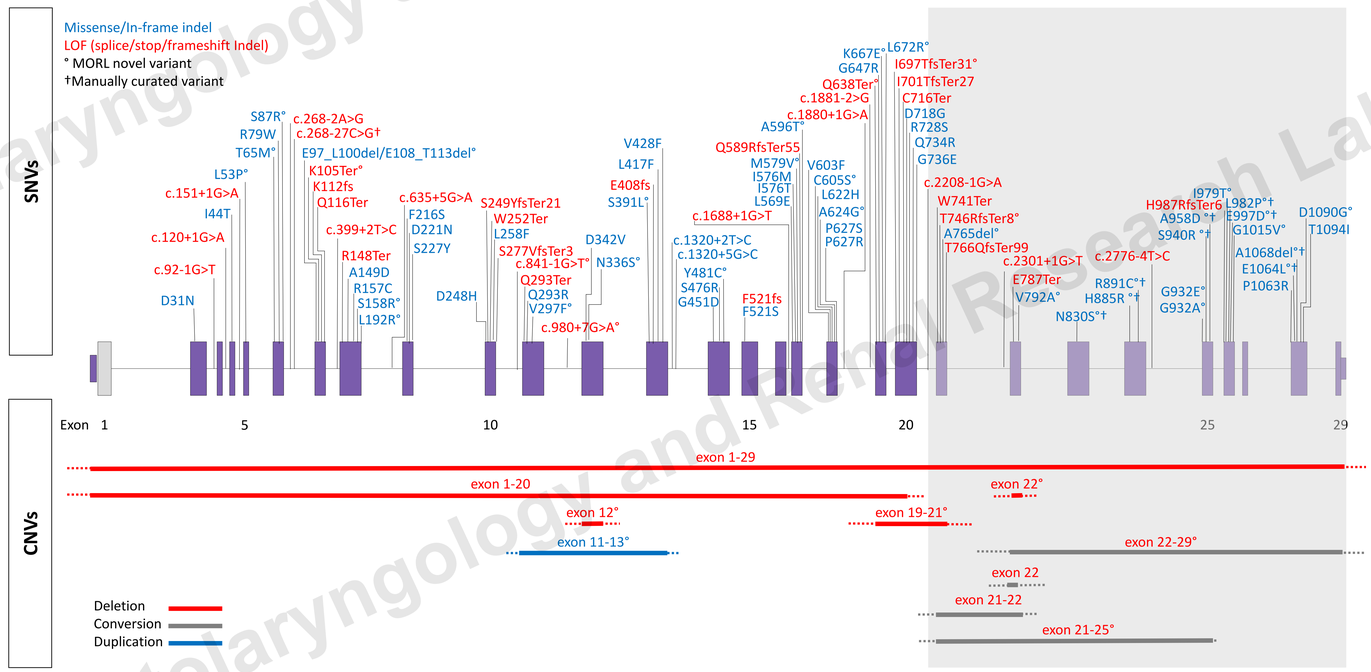


Figure 4. Causative CNVs and SNVs in *OTOA* found within the MORL cohort and published literature. 100 causative SNVs were identified in *OTOA*. Variants in the highly homologous region (grey box) were often filtered out by the bioinformatic pipeline due to mapping ambiguity and quality. Manual curation using Integrated Genomics Viewer (IGV) was required. Bold bars indicate confirmed CNVs and dotted line denotes unknown breakpoints. All variants are mapped to transcript NM_144672.4.

Representative CNVs

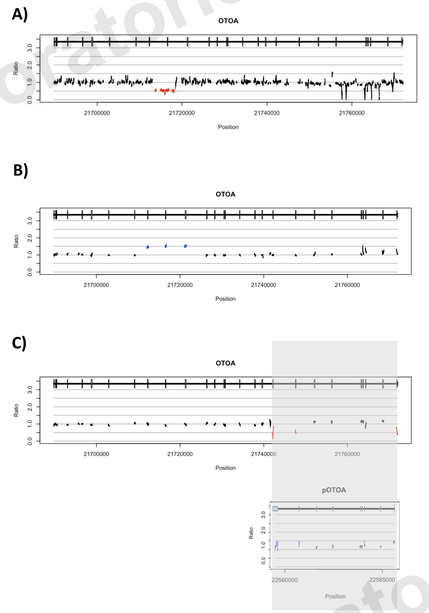


Figure 5: Representative CNVs in MORL cohort. A) Heterozygous partial deletion of exon 12. **B)** Heterozygous partial gene duplication of exons 11-13. **C)** Heterozygous gene-to-pseudogene conversion of exon 21-22 with decreased read depth in *OTOA* (top) and corresponding increased read depth in *OTOAP1* (bottom).

Conclusions

OTOA-related hearing loss exhibits high mutational diversity, far beyond whole gene deletions.

- Missense variants contribute a substantial mutational burden to DFNB22.
- Inclusion of *OTOAP1* and manual review in the highly homologous region increased diagnostic yield by 23%.

Sufficient coverage of *OTOA* and *OTOAP1* for detection of CNVs, as well as manual review of variants in regions of high homology is essential to provide accurate diagnoses of *OTOA*-related hearing loss.

References and Acknowledgements

- Azaiez H, Booth KT, Ephraim SS, et al., "Genomic Landscape and Mutational Signatures of Deafness-Associated Genes". Am J Hum Genet. 2018 Oct 4;103(4):484-497. PMID: 30245029; PMCID: PMC6174355.
- Sloan-Heggen CM, Bierer AO, Shearer AE, et al., Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. Hum Genet. 2016 Apr;135(4):441-450. PMID: 26969326; PMCID: PMC4796320.

This study was supported by NIDCDs R01s DC002842, DC012049, DC017955 to RJHS.

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