Genotype-Phenotype Correlations in *DFNA5*-Related Hearing Loss

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Introduction

- Variants in *DFNA5* gene (also known as *GSDME*) are associated with autosomal dominant non-syndromic hearing loss (ADNSHL).
- *DFNA5*-related HL is typically progressive, affecting high frequencies first.
- Our findings reveal **variant-dependent differences** in aberrant splicing levels, leading us to hypothesize that ***partial loss of splicing may result in a milder HL phenotype as compared to complete loss of splicing.***

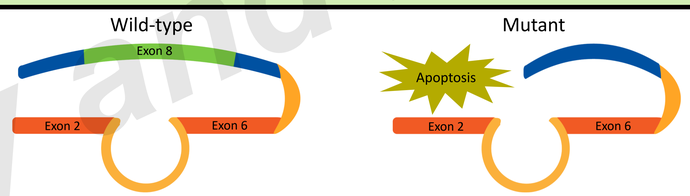

Figure 1: Schematic of wild-type and mutant DFNA5 protein. Exons 2 and 6 encode the apoptosis inducing portion of DFNA5 while exon 8 encodes part of the C-terminal domain that shields and inhibits the apoptosis-inducing domain of DFNA5. Skipping of exon 8 results in the formation of a constitutively active DFNA5 leading to apoptosis of hair cells.

Methods

- Retrospective analysis of the MORL cohort for synonymous variants in *DFNA5* exon 8
- Minigene splicing assay of seven pathogenic variants from patients previously tested for genetic deafness to determine the impact on splicing
- Literature review to identify reported pathogenic *DFNA5* variants that result in complete or partial loss of splicing
- Modeling of HL with censored mixed effects linear regression

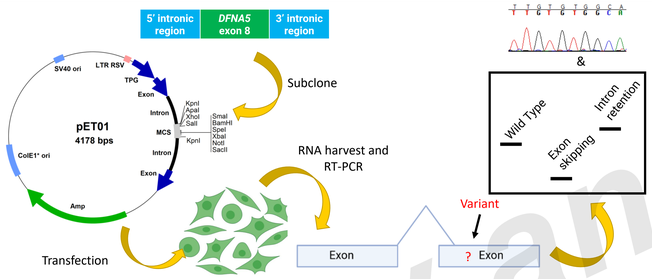

Figure 2: Overview of minigene splicing assay. *DFNA5* exon 8 and a portion of the flanking intronic regions were subcloned into a pET01 vector. Vectors were then transfected into HEK293 cells. RNA harvest and RT-PCR was performed 36-48 hrs after transfection. The impact of variants was assessed using gel electrophoresis and Sanger sequencing.

Minigene Splicing Assay

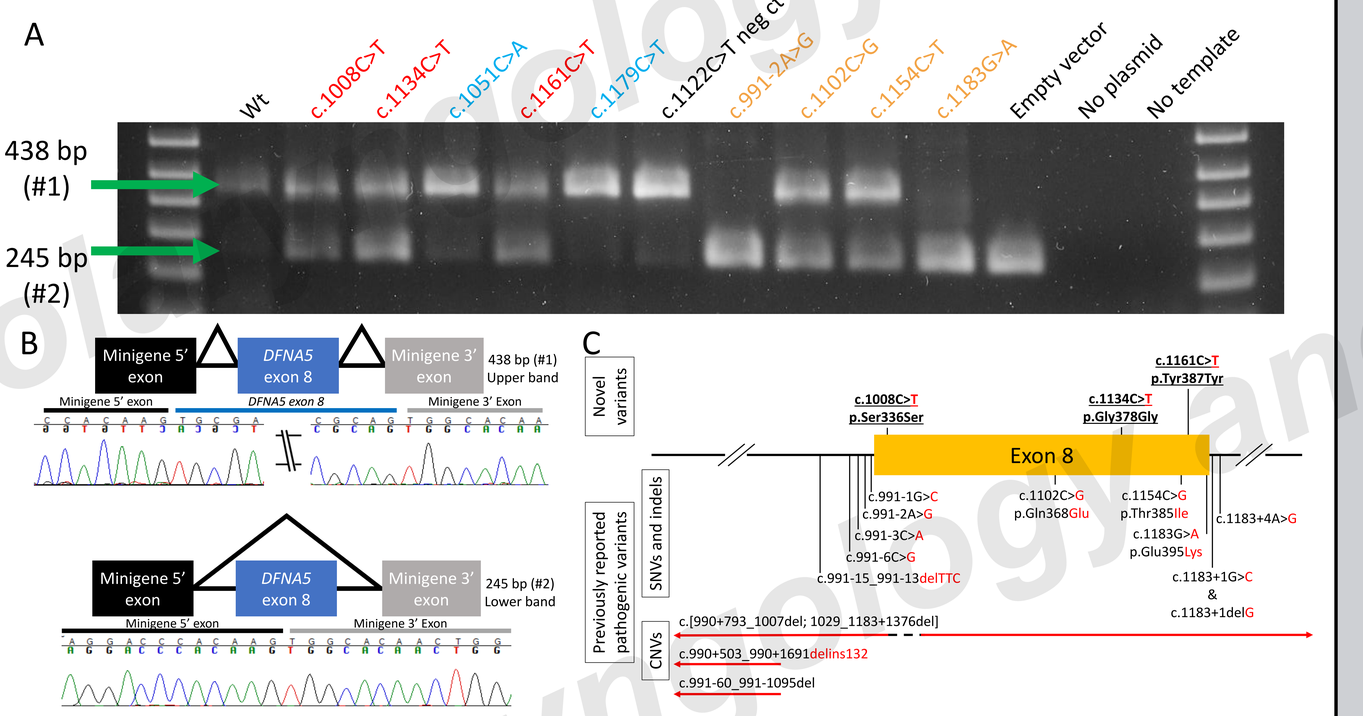

Figure 3: Variability in splicing efficiency amongst different pathogenic *DFNA5* variants. (A) Minigene splicing assay results visualized on gel electrophoresis for wild-type and mutant constructs. Three novel pathogenic synonymous variants (in red) and four previously reported pathogenic variants (in orange, as positive controls) were analyzed. The c.1008C>T, c.1134C>T, and c.1161C>T synonymous variants, along with two of the previously reported variants, resulted in partial loss of splicing, as indicated by the presence of both an upper band (438 bp, #1) and a lower band (245 bp, #2). (B) Schematic representation and sequencing of the upper band (containing exon 8) and the lower band (lacking exon 8). (C) Summary schematic of known pathogenic *DFNA5* variants. Novel variants are depicted at the top, while previously reported variants are shown at the bottom.

Audiometric Analysis

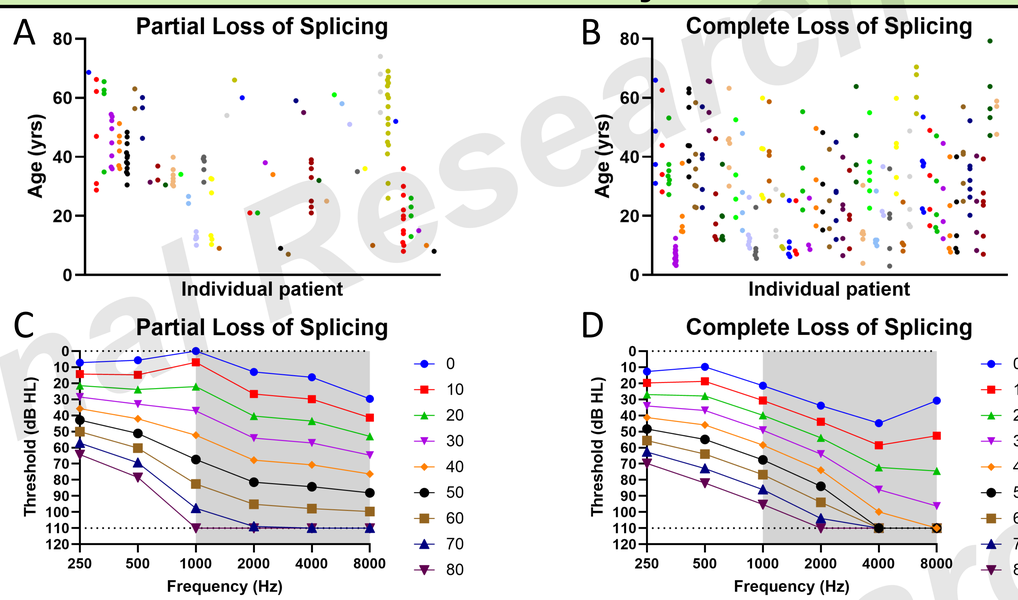

Figure 4: Severity and progression rate of HL correlate with splicing efficiency. (A-B) Dot plots showing the per patient distribution of audiograms by age for cases of (A) partial and (B) complete loss of splicing. (C-D) Age-Related Typical Audiograms (ARTAs) showing the progression of hearing loss for cases of (C) partial and (D) complete loss of splicing. Frequencies with a significant difference in either the rate of progression and/or baseline hearing are shaded in gray.

Summary Statistics

Frequency	Parameter	Adjusted p-value
250	Intercept	1.000
250	Age	1.000
500	Intercept	1.000
500	Age	1.000
1000	Intercept	**2.665e-13**
1000	Age	**2.687e-10**
2000	Intercept	**1.501e-06**
2000	Age	**9.418e-04**
4000	Intercept	**4.433e-09**
4000	Age	1.000
8000	Intercept	1.000
8000	Age	**1.461e-05**

Table 1: Censored mixed effects linear regression. The differences in HL severity and progression were statistically significant between partial and complete loss of splicing.

Conclusions

- We expand the mutational landscape of *DFNA5*-related HL to include synonymous variants.
- We show the importance of assessing persons with hearing loss for splice-altering synonymous variants, regardless of the location within the exon.
- The phenotype-genotype correlation in *DFNA5*-related HL reflects the amount of expressed mutant protein.
- Greater amounts of mutant protein result in more severe and rapidly progressing HL in the mid- to high- frequencies.

References and Acknowledgments

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