Identification of the First Pathogenic Synonymous *DFNA5* Variant

Joseph J. Chin[1,2], Amanda Schaefer[2], Diana Kolbe[2], Kevin T. Booth[3], Hela Azaiez[2], Richard J. H. Smith[2]

[1]University of Iowa Carver College of Medicine, [2]University of Iowa Hospitals & Clinics, Molecular Otolaryngology & Renal Laboratories, [3]Department of Medical and Molecular Genetics, Indiana School of Medicine, Indianapolis, IN, 46202, USA

Introduction

- Variants in the gene *DFNA5* (also known as *GSDME*) are associated with autosomal dominant non-syndromic hearing loss (ADNSHL).
- *DFNA5*-related HL is typically progressive, affecting high frequencies first.
- Currently, all known pathogenic *DFNA5* variants affect the splicing of exon 8. These variants include intronic, canonical splice site, missense, and copy number variations.

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

- Persons were tested using OtoSCOPE®, a comprehensive genetic testing platform.
- Variants were filtered and prioritized based on minor allele frequency, *in-silico* predictions, family history, and clinical correlation.
- Candidate variants were assessed using minigene splicing assays.

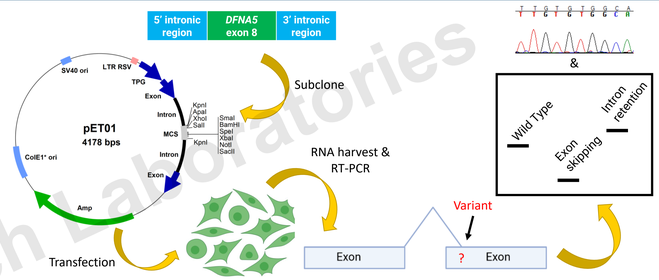
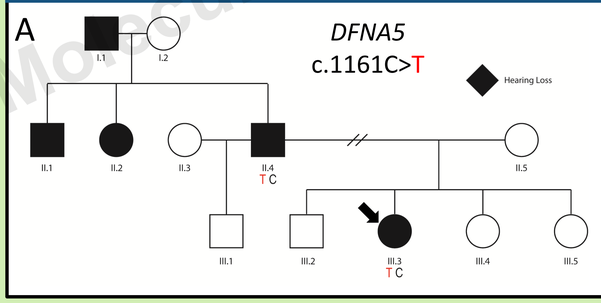
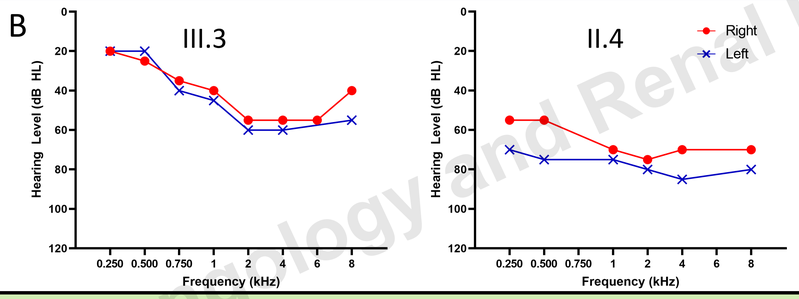


Figure 2: Overview of minigene splicing assay. *DFNA5* exon 8 and a portion of the flanking intronic regions was subcloned into a pET01 vector. Vectors were then transfected into HEK293 cells. RNA harvest and RT-PCR was performed 36-48 hrs after transfection. The impact of the variants was assessed using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

Segregation Analysis





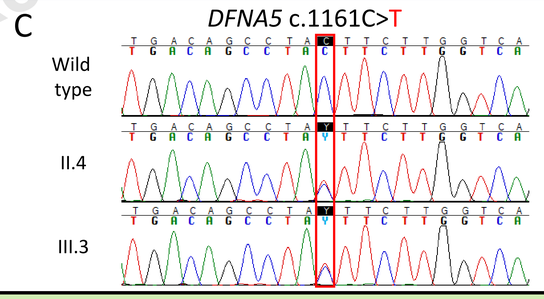


Figure 3: Segregation analysis. (A) Pedigree showing the segregation analysis of the *DFNA5* c.1161C>T variant. (B). Audiograms were obtained from the proband (III.3) at age 9 and her father (II.4) at age 41. The father has a history of middle ear infections. (C) Sanger validation confirmed that the proband (III.3) and the father (II.4) carry the variant.

In-silico assessment

A

Genomic position	cDNA position	Distance from end of exon	Presumed Protein effect
7:g.24745825G>A	c.1161C>T	22	p.(Tyr387Tyr)

gnomAD MAF	CADD	HSF prediction	SpliceAI prediction
novel	7.4	splice altering	not splice altering

B

Splice regulatory protein	Effect: Silencer (−) Enhancer (+)	Motif
9G8	−	GCCTAT
PESE	−	CCTATTTC
EIE	−	CTATTT
SRp55	−	TATTTC
PESS	+	TATTTCTT
SRp40	−	TTTCTTG

Human	GCTGTTTATGACAGCCTACTTCTTGGTCAGTGCCCTCGCAGGTAAGGA
Cat	GCTCTTTTCTACAGCCTACTTCTTGGTCAGTGCACTAGCAGGTATGGA
Chimpanzee	GCTGTTTATGACAGCCTACTTCTTGGTCAGTGCCCTCGCAGGTAAGGA
Cow	ACTCTTCTCTACAGCCTACTTCTTGGTCAGTGCGCTAGCAGGTATGGA
Crocodile	ACTCTTCTCAGCTGCTCACCTCTTGATCAGTGCTGTAGCAGGTATGTC
Dog	GCTCTTTTCTACGGCCTACTTCTTGGTCAGTGCACTAGCAGGTACGGA
Duck	ATTGGTCTCAGCTGCTCACCTCCTCATCAGTGCTCTCTCTGGTATGTT
Ferret	GCTCTTTTCTACAGCCTACTTCCTGGTCAGTGCACTAGCAGGTATGGA
Goat	ACTCTTCTCTACAGCCTACTTCTTGGTCAGTGCGCTAGCAGGTATGGA
Golden eagle	ACTCTTCTCAGCTGCTCACCTCCTTATCAGTGCTATCTCTGGTATGTT
Gorilla	GCTGTTTATGACAGCCTACTTCTTGGTCAGTGCCCTCGCAGGTAAGGA
Hamster	GCTCTTTGCAACAGCCTACTTCCTGGTCAGTGCACTAGCAGGTATGGA
Mouse	GCTCTTTGCAACAGCCTACTTCCTGGTCAGCGCACTAGCAGGTATGGA
Rabbit	GCTCTTTGCCACGGCCCACTTCTTGGTCAGCGCACTGGCAGGTATGGA
Rat	GCTCTTTGCAACAGCCTACTTCTTGGTCAGTGCACTAGCAGGTATGGA
Sheep	ACTCTTCTCTACAGCCTACTTCTTGGTCAGTGCGCTAGCAGGTATGGA
Tortoise	ACTCTTCTCAGCTGTTCACATCCTGATCAGTGCAGTGGCAGGTACAAT

Figure 4: *In-silico* assessment of the *DFNA5* c.1161C>T variant. (A) The variant is novel but is not predicted to be deleterious by CADD. (B) Assessment of changes in exonic splicing motif signal sequences and conservation. The c.1161C>T variant resides in a highly conserved region. Human Splicing Finder indicates the creation of 5 exonic splice silencers and the creation of 1 exonic splice enhancer.

Minigene Splicing Assay

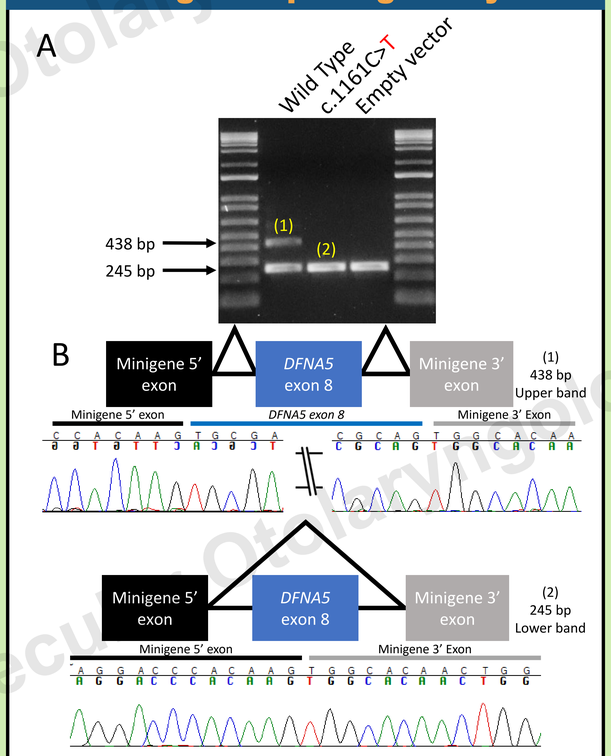


Figure 5: Minigene splicing assays. (A) Gel electrophoresis of the wild type and the c.1161C>T mutant vectors. (B) Schematic drawing and sequencing of the impact of the c.1161C>T variant. The upper (438 bp) band (#1) includes exon 8 whereas the lower (245 bp) band (#2) skips exon 8. The c.1161C>T variant results in skipping of exon 8.

Genomic Landscape of *DFNA5*

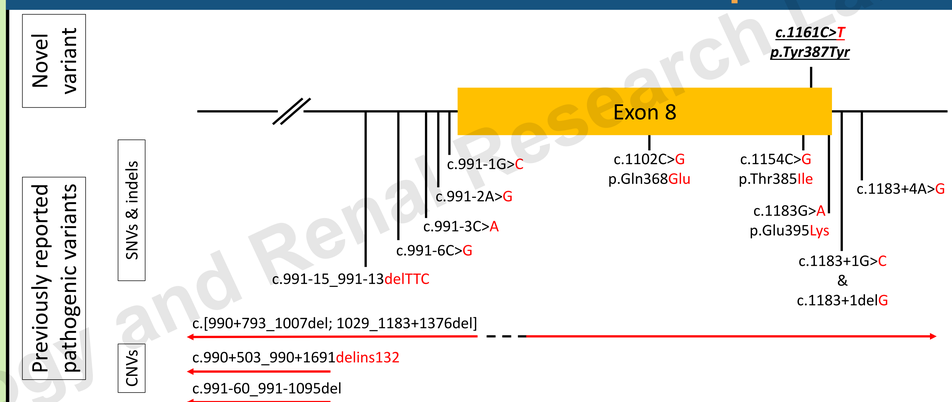


Figure 6: Schematic of known pathogenic *DFNA5* variants. There are 14 previously reported variants. Of these 14 previously reported variants, 3 are missense variants, 10 involve the intronic regions flanking exon 8, and 1 is a complex CNV.

Conclusions

- We have identified the first deafness-causing synonymous variant in *DFNA5*; c.1161C>T.
- We expand the mutational landscape of *DFNA5*-related HL to include synonymous variants.
- It is important to assess persons with hearing loss for splice-altering synonymous variants regardless of CADD score, location within the exon, or negative SpliceAI prediction.

References and Acknowledgments

1. Azaiez H, … Smith RJH. Genomic Landscape and Mutational Signatures of Deafness-Associated Genes. The American Journal of Human Genetics. 2018 Oct 4;103(4):484–497.
2. Booth KT, … Smith RJ. Exonic mutations and exon skipping: Lessons learned from *DFNA5*. Hum Mutat. 2018 Mar;39(3):433–440. PMCID: PMC5805621
3. de Beeck KO, Van Laer L, Van Camp G. *DFNA5*, a gene involved in hearing loss and cancer: a review. Ann Otol Rhinol Laryngol. 2012 Mar;121(3):197–207. PMID: 22530481
4. Mansard L, … Roux AF. Identification of the First Single *GSDME* Exon 8 Structural Variants Associated with Autosomal Dominant Hearing Loss. Diagnostics (Basel). 2022 Jan 15;12(1):207. PMCID: PMC8774889
5. Thorpe RK, … Smith RJH. AudioGene: refining the natural history of *KCNQ4, GSDME, WFS1,* and *COCH*-associated hearing loss. Hum Genet. 2022 Apr;141(3–4):877–887. PMCID: PMC9092196

This study was supported in part by NIDCDs R01s DC002842, DC012049 and DC017955 and NIGMS T32 GM139776.

Please send questions to Joseph Chin: Joseph-Chin@uiowa.edu
https://morl.lab.uiowa.edu/