The value of mitochondrial genome analysis and approaches for difficult-to-sequence regions in hereditary hearing loss panel testing

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Introduction

- Hereditary hearing loss (HHL) is a genetically heterogeneous group of disorders varying in severity, age of onset, and inheritance pattern.
- Identifying the molecular diagnosis is important in the management of patients with HHL and is considered a standard part of the evaluation.
- Both nuclear and mitochondrial genes are associated with HHL.
- Many key HHL genes, such as *STRC* and *OTOA* are difficult to sequence (DTS) and may have limitations/be excluded from available panels.
- Here we report our experience with patients with HHL based on simultaneous sequence and copy number variant (CNV) analysis of the nuclear and mitochondrial genomes combined with customized analysis for difficult-to-sequence regions in genes such as *STRC* and *OTOA*.

Methods

- 898 consecutively referred patients for the indication of HHL were tested with one of three panels: Comprehensive Hearing Loss and Deafness, Non-Syndromic Hearing Loss, or Syndromic Hearing Loss.
- 305 panel reports including the mitochondrial genome were examined.
- NGS testing was done with either NextSeq or NovaSeq technology and included up to 112 custom deep intronic variants depending on the panel. The target regions included 17 genes in DTS regions with segmental duplication (SD).
- CNVs were called from NGS data.
- Variant interpretation was performed according to ACMG guidelines.
- A diagnosis was defined as one P/LP variant in AD/XL conditions or two P/LP variants in a single AR gene.

Results

- The majority of patients were tested with the Comprehensive Hearing Loss and Deafness Panel (n=770), followed by the Non-Syndromic Hearing Loss Panel (n=106), and the Syndromic Hearing Loss (n=22).
- The median percentage of target regions covered at 20X or greater was 99.92% while the median sequencing depth was 195X.

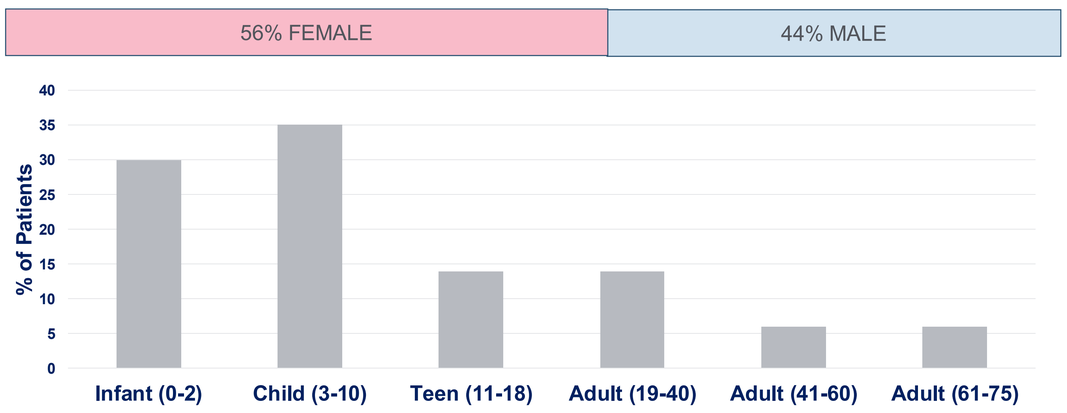

Figure 1. Distribution of age and sex in the cohort.

Diagnostic Yield

- Of 898 patients, 269 (30.0%) patients received a molecular diagnosis in a nuclear gene.
- Diagnoses were made in 54 nuclear genes.

GENE	% of diagnoses
GJB2/GJB6	24.9
STRC	9.7
SLC26A4	4.5
MYO15A	4.1
TMPRSS3	3.7
USH2A	3.7
MYO7A	3.0
CDH23, LOXHD1, MITF, TMC1	2.6 each
CHD7, TECTA	2.2 each
ACTG1, ADGRV1, MYO6, PCDH15	1.9 each
EYA1, GATA3, PDZD7, POU4F3	1.5 each
OTOA, OTOGL, WFS1	1.1 each
COL11A1, COL4A3, FGFR3, ILDR1, POU3F4, SIX1, SOX10, TCOF1, TFAP2A, TRIOBP	0.7 each
ATP6V1B1, CIB2, CLDN14, CLRN1, COL11A2, COL4A4, DFNA5, DIAPH1, EDNRB, GRXCR1, KCNQ4, LRTOMT, MPZL2, NDP, OTOF, PEX1, POLR1D, PRPS1, SLC17A8, SYNE4	0.4 each

Table. Percentage of diagnoses attributed to each gene.

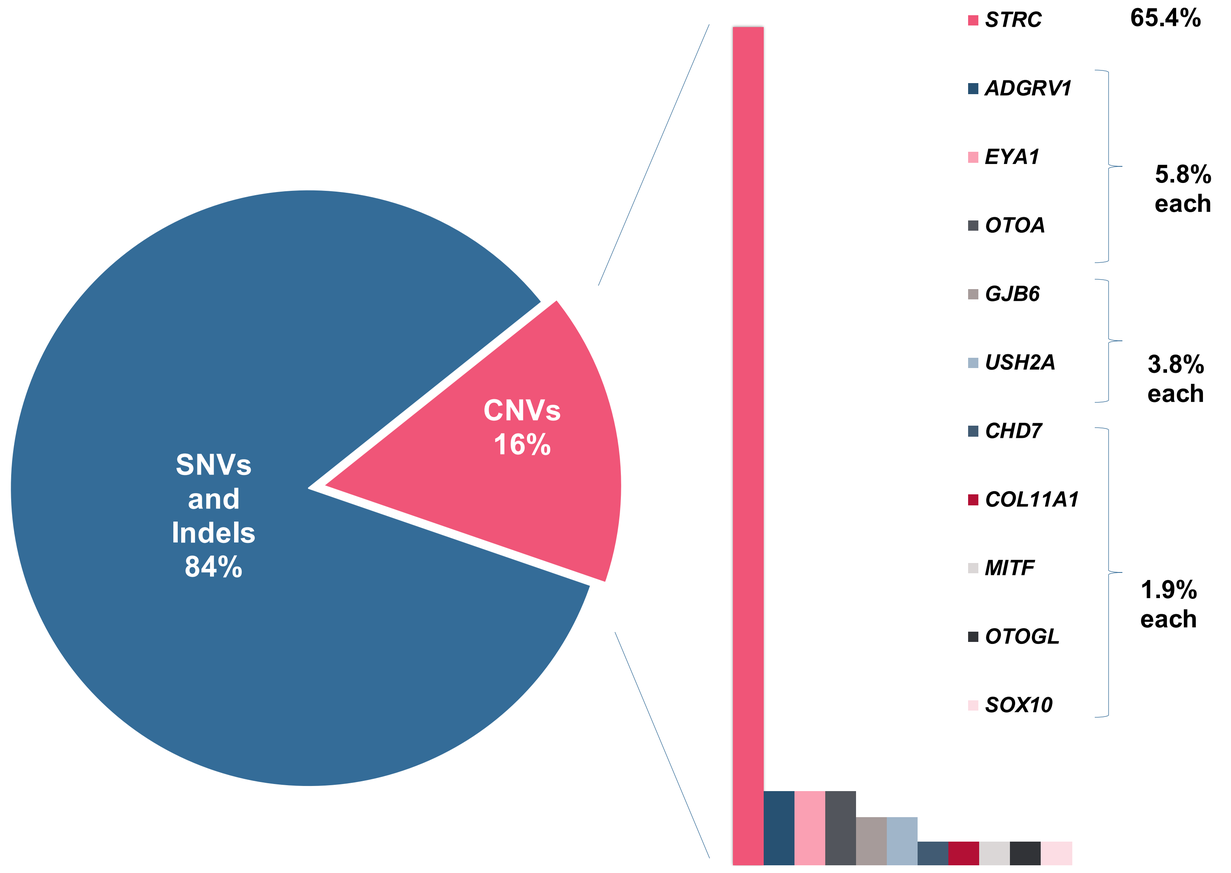

Figure 2. Percentage of diagnoses involving a CNV; frequency of genes with diagnostic CNVs.

- CNVs represent 16% of molecular diagnoses.
- Of the diagnostic CNVs, 42.2% (19/45) were intragenic deletion with 22.2% (10/45) were <4 exons in size.
- DTS genes with regions of SD contributed to 14.5% diagnoses, confirmed with orthogonal methods.
- An additional 2% diagnostic yield (6/305) was identified when the mitochondrial genome was included in hearing loss panels.

STRC Custom Analysis

- Analysis of *STRC* is complicated by very high sequence homology to its pseudogene, *STRCP1* (Figure 3).
- Deletions detected by our CNV detection algorithm were confirmed by quantitative PCR or digital PCR targeting unique regions in *STRC*.
- Sequence variants were confirmed by Sanger sequencing with primers that specifically amplify either *STRC* or *STRCP1.*

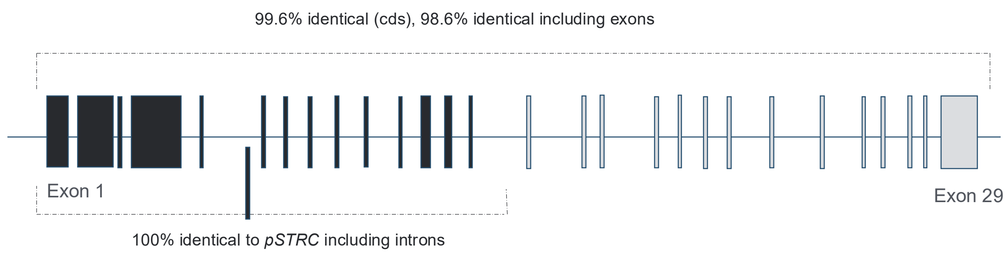

Figure 3. Schematic of *STRC* and homology to *STRCP1*. (adapted from Mandekler et al. The Journal of Molecular Diagnostics. 16(6):639-647, 2014)

Conclusions

- This study provides evidence that inclusive testing strategy with coverage of difficult-to-sequence regions and mitochondrial genome is needed to maximize diagnostic benefit for individuals with hereditary hearing loss.
- Inclusion of difficult-to-sequence genes, such as *STRC* and *OTOA,* contributes to >10% of the diagnostic yield in our cohort.
- Mitochondrial genome analysis contributes an additional 2% to the diagnostic yield.
- High quality CNV analysis, including the ability to detect small intragenic CNVs, is essential for maximized diagnostic yield.

Conflict of interest statement: All authors are employed by Blueprint Genetics, a Quest Diagnostics company.