"In-depth analysis using Next-generation Sequencing (NGS) and bioinformatics results in pediatric ADGRV1 compound heterozygotes"

Sloane Clay BS, Adele Evans M.D. FAAP, Chindo Hicks PhD, David Otohinoyi MD, Fern Tsien PhD

Louisiana State University Health Sciences Center and Children's Hospital New Orleans

Introduction

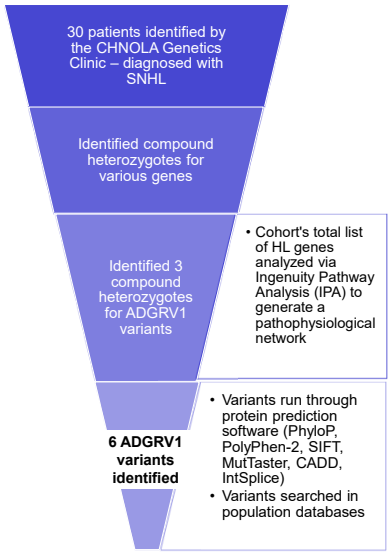
BACKGROUND: Medical evaluation of sensorineural hearing loss (SNHL) is critical to anticipatory guidance, particularly important with syndromic and/or progressive disorders like Usher syndrome (SNHL and retinitis pigmentosa). Genetic diagnosis using next generation sequencing (NGS) proves challenging with multigenic, multiallelic variants of uncertain significance (VUS). Identification and reclassification of VUS is a fluid process, accelerated by genetic testing companies, and thus necessitates in depth-analysis and interpretation. Compound heterozygous variants may be misinterpreted as benign but in fact result in deleterious combined autosomal recessive expression, important to diagnosis.

Variants in ADGRV1 (inherited in a recessive or compound heterozygous manner) have been implicated in Usher Syndrome (Type II). Usher Syndrome Type II presents as SNHL at birth and retinitis pigmentosa (RP) in the second decade of life leading to blindness.

SIGNIFICANCE: If variants in genes like ADGRV1 can be identified leading to earlier diagnoses, then patients can receive access to learning resources before their visual and/or hearing impairment becomes too severe.

HYPOTHESIS: The ADGRV1 variants present in the cohort contribute to their clinical presentation, along with other genes in which they carry VUS.

Figure 1: Approach



CHNOLA – Children's Hospital New Orleans, SNHL – sensorineural hearing loss

Figure 2: ADGRV1 Cohort Pedigrees

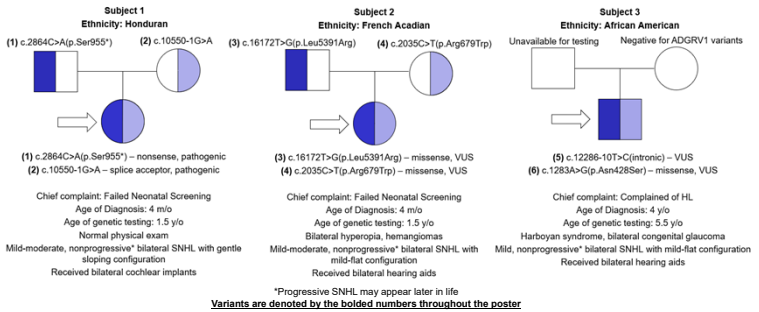
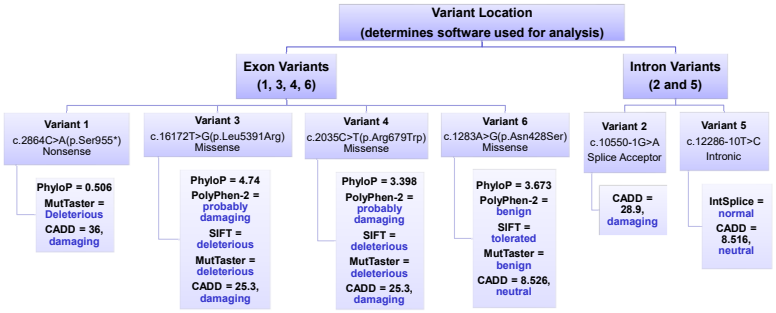
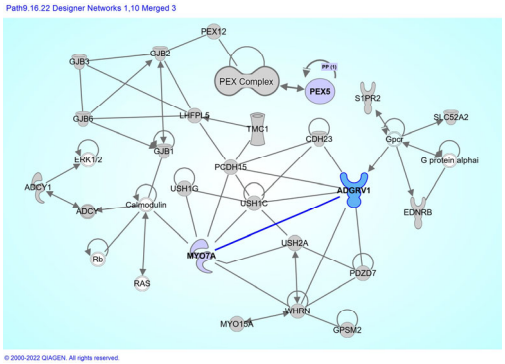


Figure 3: ADGRV1 Variant Prediction



The software programs MutTaster, PolyPhen-2, and SIFT perform in-silico analysis of exonic variants and predicts their effects on the protein product.
CADD is a pathogenicity score based on functional and evolutionary data (low score = neutral; high score = damaging)
PhyloP score represents the evolutionary conservation of the nucleotide; a positive score means that nucleotide is conserved. Higher scores are considered more deleterious.
IntSplice predicts a splicing consequence of a single nucleotide variation (SNV) at intronic positions -50 to -3 close to the 3' end of an intron of the human genome

Figure 4: IPA Network – Ingenuity Pathway Analysis



IPA creates networks based on gene function → does not account for variants within genes

All genes present in this network are associated with hearing loss. Subjects contain variants in the colored genes.

Symbols denote the type of molecule the gene encodes.
ADGRV1 = blue, transmembrane receptor
MYO7A = purple, enzyme
PEX5 = purple, Other (peroxisomal targeting signal 1 receptor)

The network demonstrates a direct binding association between MYO7A and ADGRV1. Both genes are implicated in Usher Syndrome Type II

Results

We identified six different ADGRV1 variants in three ethnically diverse families.

Protein Prediction Summary (Figure 3)

- **Subject 1:** Both variants (1 and 2) predicted as "damaging or deleterious", consistent with current classification.
- **Subject 2:** Both variants (3 and 4) predicted as "damaging or deleterious".
- **Subject 3**: Both variants (5 and 6) predicted as "benign or neutral".

IPA Analysis Results (Figure 4)

- The network demonstrates a direct binding relationship between ADGRV1 and MYO7A.
  - This relationship is supported by the literature. Both genes are involved in Usher Syndrome (Type II).
  - Subject 2 has a MYO7A variant along with 2 ADGRV1 variants.
  - The implications of Subject 2's variants on ADGRV1-MYO7A binding require further investigation.
- The network demonstrates PEX5 operating in an adjacent network to ADGRV1 and MYO7A.
  - Subjects 2 and 3 both have variants in PEX5.
  - The implications of Subject 2 and 3 variants on the larger network requires further investigation.

ADGRV1 Variants - Population Database Results (Figure 5)

Each ADGRV1 variant in our cohort was cross-referenced with 3 population databases (ExAD, gnomeAD, and 1000genome).

Subject 1 - Honduran (Variants 1 and 2)	(1) Previously reported, but not in Latin American population (2) Not reported to date
Subject 2 – French Acadian (Variants 3 and 4)	(3) Not reported to date (4) Previously reported, but not in French/Cajun population
Subject 3 – African American (Variants 5 and 6)	Both (5) and (6) previously reported in the African American population

Conclusion

- VUS should not be misinterpreted as "benign".
  - Especially true of compound heterozygotes because diseases like Usher Syndrome are typically characterized as "autosomal recessive" disorders.
- Analysis of VUS using open-source bioinformatics software supports clinicians and researchers in VUS reclassification.
  - Our data will be submitted to national genetics databases. As the number of submissions for specific variants grows, the American College of Genetics may reclassify VUS based on the available data.
- It is vital for clinicians to follow up regularly with these patients as variant reclassification is occurring rapidly.
- VUS reclassification enables physicians to provide better anticipatory guidance, ultimately leading to high-quality healthcare and improved academic support for diverse special-needs populations.
- Further investigation regarding the compounding effects of variants (especially VUS) in hearing loss genes must be done in order to understand their potential combined clinical impact.

This research project was supported through the LSU Health Sciences Center, School of Medicine.