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

Genetic diagnosis of sensorineural hearing loss (SNHL) using NGS proves challenging when encountering multigenic, multiallelic variants of uncertain significance (VUS). These VUS make it difficult to provide anticipatory guidance regarding progressive disorders such as Type II Usher syndrome (SNHL at birth and retinitis pigmentosa in the second decade of life). With genetic testing companies are identifying, reporting, and reclassifying VUS at a rapid pace; there is a need for in depth-analysis and interpretation. For example, VUS in ADGRV1, a gene implicated in Type II Usher Syndrome, may be inherited in a compound heterozygous manner and misinterpreted as benign, when in reality their combined expression leads to an affected patient. The purpose of this retrospective study was to assess ADGRV1 compound heterozygotes with VUS in other genes and to predict the role of identified ADGRV1 variants in the subjects' SNHL.

After IRB review and exemption, the Children's Hospital Genetics Clinic provided a list of 30 patients diagnosed with SNHL who underwent genetic testing via the Invitae Deafness panel between 2017-2022. A cohort of 3 ADGRV1 compound heterozygotes (H90.3) was formed based on the availability of audiograms, pedigrees, and parental genetic testing results. 6 ADGRV1 variants were tested using PhyloP, MutTaster, SIFT, Polyphen-2, CADD, and IntSplice to predict variant pathogenicity. The variants were cross-referenced in ExAC, genomAD, and 1000 genome population databases. Finally, the Invitae Deafness Panel gene list was uploaded to Ingenuity Pathway Analysis (IPA) to visualize pathophysiological relationships between ADGRV1 and other altered genes seen in the cohort. All data was stored in encrypted files.

Six different ADGRV1 variants in three ethnically diverse families were identified. Subject 1 (Honduran) carried two known pathogenic variants (c.2864C>A(p.Ser955*), c.10550-1G>A). The prediction software supported the current classification of these variants. Subject 2 (French Acadian) carried two VUS (c.16172T>G(p.Leu5391Arg), c.2035C>T(p.Arg679Trp)), predicted as damaging/deleterious. Subject 3 (African American) carried two VUS (c.12286-10T>C (intronic), c.1283A>G(p.Asn428Ser)), predicted as benign/tolerated.

Analyzing variants using bioinformatics software allows clinicians and researchers to identify VUS that warrant further functional investigation. As VUS are reclassified, physicians can provide better anticipatory guidance and appropriate interventions, ultimately leading to improved quality of life for diverse special-needs populations.