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Using the Phenome and Genome to Improve Genetic Diagnosis for Deafness

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The advent of massively parallel sequencing (MPS) has revolutionized genetic testing for deafness by enabling personal genomics in diagnosis (for a comprehensive review, see Shearer et al¹). This technology has drastically increased the throughput of genetic testing but concomitantly has exponentially increased the amount of genetic data generated. To address this deluge of data and to streamline analysis, we have developed a custom variant prioritization pipeline incorporating data from a patient's genome and phenome (the patient's phenotype). In aggregate, the patient's phenome is his or her constellation of phenotypic traits, which for hearing loss includes the patient's audioprofile (pattern of hearing loss on audiogram), temporal bone anatomy (imaging), and ocular pathology (fundoscopy). Here we present 3 cases to illustrate how knowledge of a patient's phenome can assist variant prioritization by corroborating likely pathogenic variants and excluding variants of unknown significance (VUS).

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Author Contributions

Robert W. Eppsteiner, designed research, analyzed data, wrote manuscript, performed research; **A. Eliot Scherer**, designed research, analyzed data, wrote manuscript, performed research; **Michael S. Hildebrand**, designed research, analyzed data, performed research; **Kyle R. Taylor**, designed research, analyzed data, performed research; **Adam P. DeLuca**, designed research, analyzed data, performed research; **Steve Scherer**, performed research; **Patrick Huygen**, designed research, analyzed data; **Todd E. Scheetz**, designed research, analyzed data; **Terry A. Braun**, designed research, analyzed data; **Thomas L. Casavant**, designed research, analyzed data; **Richard J. H. Smith**, designed research, analyzed data, wrote manuscript.

Competing interests: None.

Methods

Patients and Phenomic Data

All methods were approved by the institutional review board at the University of Iowa. Clinical data were collected from all patients, including audiometric data, family history, and temporal bone imaging. Included patients segregated autosomal dominant hearing loss, and environmental causes were ruled out. Audiometric profiling (using air conduction thresholds) was performed using AudioGene v4.0, freely available at <http://audiogene.eng.uiowa.edu>, which includes 3246 audiograms from 1445 patients that map to 34 loci.^{2,3}

Genomic Data and Bioinformatics Analysis

DNA was extracted from peripheral blood, and OtoSCOPE testing (<http://morl-otoscope.org>) was performed as described previously.⁴ All exons of all genes implicated in nonsyndromic hearing loss (NSHL) and NSHL mimic genes (those causing Usher and Pendred syndromes) were targeted for enrichment and sequenced on the Illumina HiSeq (Illumina, Inc, San Diego, California). Sequencing reads were mapped with BWA and variants were called using the GATK Unified Genotyper. Variants were filtered against (1) a list of all known pathogenic changes to identify any previously implicated deafness-causing mutations (<http://deafnessvariationdatabase.org>), (2) publicly available population-scale genomic databases (ESP, 1000Genomes), and (3) an internal variant list that includes data from all prior OtoSCOPE runs. On average, <10 VUSs remain per patient. Clinically relevant variants were confirmed with Sanger sequencing.

Results

Patient 1

Patient 1 is a 66-year-old man with dominant NSHL and a progressive, “up-sloping” audioprofile (Table 1 and Figure 1A). OtoSCOPE variant analysis identified 1 reported pathogenic variant (*WFS1* p.R685P) and 2 high-quality VUSs. AudioGene predicted DFNA6 (*WFS1*) as the most likely affected loci. Filtering the OtoSCOPE-generated variant list against AudioGene data corroborated this reported variant, *WFS1* p.R685P, as causative and allowed the exclusion of the other VUSs.

Patient 2

Patient 2 is a 57-year-old man with dominant NSHL displaying a down-sloping audioprofile (Table 1 and Figure 1B). Genetic testing with OtoSCOPE identified 3 VUSs and a reported pathogenic mutation in *KCNQ4* (p.L281S). AudioGene predicted DFNA2 (*KCNQ4*) as the most likely cause of the patient’s audioprofile. Concurrence between OtoSCOPE and AudioGene data allowed exclusion of VUSs.

Patient 3

Patient 3 is a 55-year-old woman with dominant NSHL and a sharply down-sloping audiogram (Table 1 and Figure 1C). Genetic testing with OtoSCOPE identified 6 VUSs. AudioGene predicted DFNA2 (*KCNQ4*), DFNA9 (*COCH*), and DFNA25 (*SLC17A8*) as likely causes of hearing loss. No coding variants were identified in *KCNQ4* or *COCH*; however, 1 VUS was identified in *MYO7A*. The *MYO7A* variant, p.R1743W, is novel and predicted to be pathogenic by several in silico pathogenicity prediction algorithms (BLOSUM62, SIFT, PolyPhen2, and PhyloP). DFNA11 was not predicted by audiogene to be causative. However, this may reflect a limitation to the support vector machine (SVM): it has been trained on a set of audioprofiles and is inherently limited by the number of

individual audioprofiles per genotype and phenotype. Upon manual comparison with a previously published DFNA11 mutation, the audioprofiles closely matched.⁵ As we ascertain more audioprofiles, the SVM will improve in accuracy. Sanger sequencing confirmed the presence of the *MYO7A* p.R1743W variant in patient 3.

Discussion

The capacity of massively parallel sequencing technology is vast, as a single run on the Illumina HiSeq generates more sequencing data than the entire human genome project did per year using the previous generation of sequencing technology. As such, data interpretation has replaced sequencing as a bottleneck in genomic analysis. OtoSCOPE, for example, targets only 66 genes but yields an average of 252 genomic variants that must be categorized and prioritized. A step toward facilitating this interpretation is integrating the genome with the phenome.

AudioGene and OtoSCOPE provide a focused example of this integration. We have begun expanding our phenotypic categorization of patients with hearing loss and have used AudioGene to identify specific audioprofiles, which aid in genomic variant interpretation and can help achieve a genetic diagnosis. We demonstrate that the AudioGene tool can streamline cumbersome variant analysis by corroborating reported variants and allowing exclusion of others.

As costs decrease, comprehensive screening with multigene panels will replace individual gene testing. Our lab offers both individual gene testing and comprehensive testing with the OtoSCOPE platform, which includes 66 genes. Tiered genetic testing of individuals with apparent recessive deafness by first screening *GJB2* prior to completing a multigene screen using a panel such as OtoSCOPE may be more cost-effective currently. However, as costs continue to decrease, comprehensive testing will supplant individual gene testing for deafness.

Conclusion and Future Directions

AudioGene facilitates variant prioritization in the context of deafness. For that reason, we are expanding AudioGene to include recessive forms of deafness and additional phenotypic data, including temporal bone imaging and funduscopy, which will aid in identifying pathogenic variants in Usher and Pendred syndromes.

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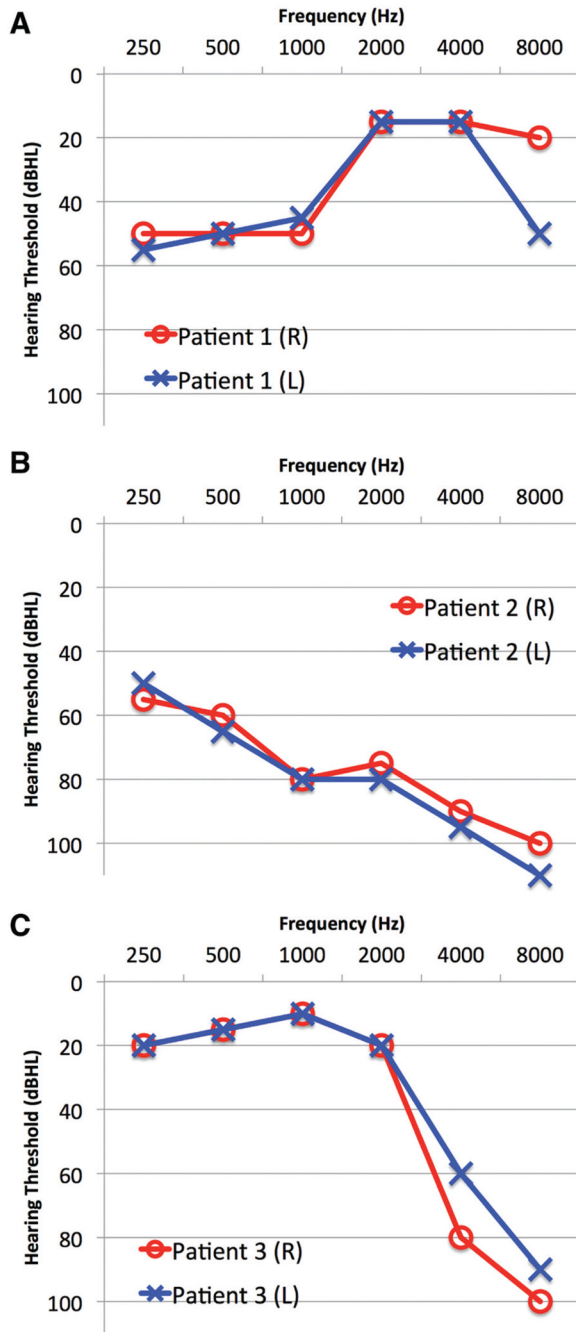


Figure 1. Audiograms from patients described in this study. (A) Patient 1 presented with a characteristic up-sloping audioprofile associated with a mutation in *WFS1* (DFNA6), (B) patient 2 presented with a down-sloping audiogram associated with a mutation in *KCNQ4* (DFNA2), and (C) patient 3 presented with a sharply down-sloping audioprofile with a causative mutation in *MYO7A* (DFNA11).

Table 1

Patients in This Study: Type of Hearing Loss, Inheritance, Genes Screened, Audiogene Prediction, and Final Result

Patient	Inheritance	Type of Hearing Loss	OtoSCOPE Result: Genes with VUS	Audiogene Prediction	Final Result
Patient 1	Dominant	Up-sloping	DSPP, LRTOMT, WFS1	DFNA6 (<i>WFS1</i>), DFNA11 (<i>MYO7A</i>), DFNA8/12 (<i>TECTA</i>)	WFS1 p.R685P
Patient 2	Dominant	Down-sloping	CDH23, GPR98, KCNQ4, WFS1	DFNA2 (<i>KCNQ4</i>), DFNA10 (<i>EYA4</i>), DFNA9 (<i>COCH</i>)	<i>KCNQ4</i> p.L281S
Patient 3	Dominant	Sharply down-sloping	MYO1A, MYO7A, OTOA, PCDH15, TRIOBP (2)	DFNA2 (<i>KCNQ4</i>), DFNA9 (<i>COCH</i>), DFNA25 (<i>SLC17A8</i>)	<i>MYO7A</i> p.R1743W

Abbreviation: VUS, variants of unknown significance.