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Navigating genetic diagnostics in patients with hearing loss

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Abstract

Purpose of review—In the age of targeted genomic enrichment and massively parallel sequencing there is no more efficient genetic testing method for the diagnosis of hereditary hearing loss. More clinical tests are on the market, which can make choosing good tests difficult.

Recent findings—More and larger comprehensive genetic studies in patients with hearing loss have been published recently. They remind us of the importance of looking for both single nucleotide variation and copy number variation in all genes implicated in non-syndromic hearing loss. They also inform us of how a patient's history and phenotype provide essential information in the interpretation of genetic data.

Summary—Choosing the most comprehensive genetic test improves the chances of a genetic diagnosis and thereby impacts clinical care.

Keywords

Genetic diagnosis; hereditary hearing loss; targeted genomic enrichment; clinical testing

Introduction

Early diagnosis of hereditary hearing loss has a large impact on a patient's clinical course. A genetic diagnosis of non-syndromic hearing loss (NSHL) obviates the need for additional 'rule-out testing' or referrals, and one of syndromic hearing loss allows for early monitoring and intervention, ultimately decreasing medical spending [1, 2*]. The 2014 ACMG guidelines for diagnosis of hearing loss recommend incorporating genetic testing early in diagnostic protocols for the evaluation of NSHL. After a thorough history, physical and audiometric assessment, the next step should involve either *GJB2* and *GJB6* testing or

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Conflicts of interest

CMSH discloses no conflict of interest. RJHS directs the MORL, which offers TGE + MPS as a clinical diagnostic test (OtoSCOPE) for hearing loss.

immediate comprehensive genetic testing [3]. Although early genetic diagnosis used to be a lofty goal, it is now achievable with targeted genomic enrichment and massively parallel sequencing (TGE+MPS). TGE+MPS has revolutionized the way genetic testing is performed and promulgated advances in research and clinical testing, diagnosis and discovery, and variant interpretation. This technology has become ubiquitous and ordering options have become so plentiful that the choice of a specific platform can be complicated and difficult. In this review we discuss important factors to consider when choosing a genetic test for a patient with hearing loss, as illustrated by recent studies and discoveries.

TGE+MPS in hearing loss diagnosis and available testing options

NSHL is an extremely heterogeneous condition and the number of causal genes continues to increase. In 2015 and the first half of 2016, for instance, 14 additional genes were identified as causing NSHL in humans [4–17,18*], increasing the number of NSHL genes to nearly 100 (http://www.hereditaryhearingloss.org). Prior to TGE+MPS, the best genetic diagnosis for NSHL required serial gene-by-gene Sanger sequencing, a constraint that made comprehensive diagnostic testing prohibitively slow and expensive. TGE+MPS now facilitates comprehensive genetic diagnosis for NSHL with a single test. A recent comparison of the ordering habits of otolaryngologists and geneticists, evaluated using four case presentations, identified a correlation between clinician comfort and familiarity with genetic testing and the likelihood of ordering comprehensive genetic testing. As might be expected, geneticists were more familiar with TGE+MPS and more likely to order genetic testing, highlighting the importance for ongoing education to ensure that all clinicians understand and confidently utilize genetic testing options [2*].

As of June 2016, 12 unique labs offer molecular diagnostic testing involving TGE+MPS methods via NextGxDx (https://www.nextgxdx.com/) or on their own website (http://www.otogenetics.com/) (Table 1). This abundance of testing options creates confusion as several test-specific differences must be considered, including: 1) The genes included in analysis; 2) Variant detection methodology; 3) Copy number variation (CNV) analysis and methodology; 4) Analytic pipelines and validation; and 5) Cost and turnaround time. Additionally, it is important to consider a patient's history and phenotype before ordering genetic testing to determine the applicability of this type of testing and after generating a genetic variant list to assess the concordance of the phenotype and the genotype.

Genes included

The most important factor when choosing a test is determining if it will adequately target the genes of interest given a patient's phenotype. Each of the currently available tests (Table 1) has a unique focus and targets. Some laboratories focus on the most likely diagnoses, others are more expansive and include genes implicated in only single families, and still others include various syndromic forms of hearing loss. As an example of the first, OtoSeq targets 23 genes, which are the most common known causes of NSHL. OtoSCOPE (v7), in comparison, targets more genes (133 total) than any other panel and includes all known genetic causes of NSHL and syndromes that may initially present as NSHL (so-called NSHL

mimics). A summary of genes included in currently available tests can be found in Supplementary Table 1.

Many recent studies illustrate the importance of a more comprehensive approach. These studies show a large amount of population-specific heterogeneity. In a study of 10 Cameroonian multiplex families with autosomal recessive NSHL (ARNSHL), for example, 7 diagnoses were possible with comprehensive testing (OtoSCOPE v5, 89 genes), implicating *CDH23, LOXHD1, MYO7A, OTOF, SLC26A4* and *STRC* as causes of hearing loss [19]. Although this study is small, it is nonetheless noteworthy for the significantly higher diagnostic rate than previously possible in Sub-Saharan African studies focused on *GJB2*, a common cause of ARNSHL in many other world population groups [20].

Targeted panels can also be very helpful in the diagnosis of syndromes, especially when the phenotype is variable or subtle. In a cohort of 67 Chinese Usher syndrome patients, using a retinal panel of genes, it was possible to provide a genetic diagnosis to 49 persons (70%). An additional 10 patients carried single likely causative alleles in *USH2A* suggesting that non-coding variants are a common cause of USH2A in this population [21]. As these and other non-coding variants are implicated in hearing loss it will be essential to expand platforms to include these genetic regions [22]. This study also exemplifies the complexity of genetic testing– the authors identified a patient with concurrent DFNB2 NSHL and RP49 (*MYO7A* and *CGNA1* variants) [21]. Including possible diagnoses outside of the obvious often provides key diagnostic information to physicians for a guided clinical correlation. An excellent example is genetic testing for prelingual hearing loss. An apparent severe-to-profound NSHL at 6 months of age may in fact ‘morph’ into a syndromic form of hearing loss with age. Usher syndrome type 1 is the classic and most common NSHL mimic, although there are many others, attesting to the value of including carefully selected causes of syndromic hearing loss on NSHL panels.

Diagnostic rate

The diagnostic rate for TGE+MPS panels is ~40%, making comprehensive genetic testing the single best diagnostic test in the evaluation of hearing loss. Sommen and colleagues evaluated 131 *GJB2*-negative individuals with ARNSHL using a 79-gene panel that included in-house CNV detection and a variant scoring system; they estimated their positive diagnostic rate as ~30% [23*]. Bademci and colleagues investigated 160 multiplex *GJB2*-negative ARNSHL families for causative variants in 58 genes and identified a genetic etiology in 90 (56%) families, who segregated likely pathogenic or pathogenic variants in 31 genes [24**]. Moteki and colleagues diagnosed 27% of 194 probands in a *GJB2*-negative Japanese cohort. Their diagnoses included 20 genes and they estimated a diagnostic rate of 40% if they had not preselected their patient population [25]. They also provided an in-depth phenotypic analysis that adds to our knowledge of the clinical presentation associated with several genes, including *LRTOMT, P2RX2, POU3F4, PTPRQ*, and *USH2A* [26–30].

Amongst a cohort of 302 Iranian probands with ARNSHL, Sloan-Heggen and colleagues illustrated the genetic diversity associated with a high co-efficient of inbreeding–they diagnosed 40 different genetic causes of hearing loss in 201 of 302 (67%) patients. Twenty-

six genes had not been previously reported as a cause of ARNSHL in Iran, suggesting that it may be short sighted to restrict the genetic search space to commonly implicated deafness-causing genes, especially when diverse ethnicities are being tested [31].

The most all-encompassing study to date was completed by Sloan-Heggen and included an analysis of 1119 sequentially accrued clinically identified probands [32*]. 440 (39%) patients were diagnosed with a hearing loss secondary to variants in 49 different genes. This study used OtoSCOPE v4 (66 genes) and v5 (89 genes), with the added genes accounting for additional 2% diagnostic rate, supporting regular updates to panels to maintain comprehensive testing. Nearly 25% of diagnoses were syndromic (101), the most common being Usher syndrome. Surprisingly, many of these patients were reported to have a normal physical exam, suggesting that a focused history was not obtained and showing that Usher syndrome is frequently unrecognized. Covering NSHL mimics like Usher syndrome in diagnosis is therefore essential.

The largest comprehensive study to date, by Nishio and Usami, reported a diagnostic rate of ~40% in 1120 Japanese patients [33**]. Of the 112 genes these authors targeted with TGE +MPS panels, 30 genes were reported as deafness-causing in their cohort. In addition, of the 2631 candidate variants they identified, only 105 variants had been reported pathogenic in the Deafness Variation Database [34] or ClinVar [35]. These included variants in *ACTG1, CDH23, COCH, COL4A5, COL11A2, CRYM, EYA1, GJB2, GJB3, GJB6, KCNQ4, LOXHD1, MARVELD2, MYH9, MYO6, MYO7A, MYO15A, OTOF, SIX1, SLC26A4, TECTA, TMC1, TMIE, TMPRSS3, USH1C, USH2A*, and *WFS1*. Their list of new candidate variants, which is being published separately, attests to the requirement for ethnic-specific data and includes variants in *ACTG1, COCH, COL11A2, GRXCR1, KCNQ4, MYO6, MYO15A*, and *TMPRSS3* [36–42].

Detection methodology

Although TGE+MPS is comprehensive, Sanger sequencing is valuable in select circumstances and is often used for small targets like *GJB2, SLC26A4,* and *mtDNA* in well-defined populations or in patients meeting select phenotypic criteria. In China, for example, 33% of 484 patients were diagnosed with NSHL by screening only these three genes in the Han, Hui and Tibetan ethnicities. The respective gene-specific diagnostic rates were: *GJB2* - 17.52%, 15.35%, and 11.43%; *SLC26A4* - 12.39%, 8.84%, and 8.57%; and *mtDNA* 1555A>G - 8.97%, 3.72%, and 5.71% [43]. In contrast, the limitation of Sanger sequencing was illustrated in a screen of eight small genes (*CABP2, CIB23, DFNB59, GJB3, ILDR1, LHFPL5, LRTOMT*, and *TMIE*) in a cohort of 72 Czech patients – only 1 positive diagnosis was made [44].

Using Sanger sequencing in a diagnostic workflow based on phenotypically driven gene testing can be successful if the phenotype is well understood. Tang and colleagues [45] performed this kind of testing on 71 patients with syndromic hearing loss or auditory neuropathy and identified a likely pathogenic or pathogenic variant in 25 patients (35.2%) and a positive diagnosis in 9 (12.7%). Because hearing loss syndromes are challenging to diagnose, this type of approach requires a high degree of specialized understanding of the

genotype-phenotype correlations associated with hereditary hearing loss. Indeed, after unsuccessful phenotype-driven candidate gene screening, Kim and colleagues used TGE +MSP (80 genes) to diagnose three of six families with NSHL (*COCH* and *GJB2*) and Waardenburg syndrome (*PAX3*) [46]. This more comprehensive approach highlighted previously-unappreciated physical features of Waardenburg syndrome in the proband (synophrys and prematurely greying hair), showing that the genetic diagnosis is often disease defining and can inform a more thorough physical examination.

Microarrays provide an alternative to Sanger sequencing but offer an even narrower detection window, one limited to variants included on the chip. Nevertheless, when variants are highly enriched in a population this approach is very cost effective. For example, amongst 1164 Chinese patients with early onset severe-to-profound hearing loss, 28% had at least 1 of 8 common mutations in *GJB2, SLC26A4*, or *mtDNA* [47]. The authors then presumed a diagnosis of ARNSHL even in heterozygous cases, discounting the carrier frequency for common variants, which can be high. Within a Brazilian cohort with NSHL, evaluated using a similar platform, variants were identified in 30% of 180 patients, however the diagnostic criteria were strict and only 19% received definitive diagnosis [48].

In general, TGE+MPS with no or minimal pre-screening is now the preferred screening option, although debate over the use of a custom-designed panel or selective filtering of whole exome sequencing (WES) remains. Each year, more genes are implicated in hearing loss, most of which are identified using WES (Reviewed through 2014 in[49], [4–17,18*]). These discoveries mean that a targeted panel must be regularly updated to be comprehensive. Aside from this limitation, targeted panels offer many advantages over WES. Deafness-specific TGE panels (see Table 1) restrict analysis to likely relevant genes, decrease sequencing costs, improve sequencing quality, and ease analysis and counseling by removing the burden of many secondary findings that are identifiable in WES.

Small panels also offer better targeted coverage. Bademci et al. have shown that overall gene coverage and specifically coverage of 58 ARNSHL genes has increased with versioning of WES [24**]. While this improved performance has closed the gap between custom panels and WES, a significant difference in coverage still exists (mean gene coverage at 10X with the v5 exome capture of 88.6%, while Sloan-Heggen reported 10x coverage of 99.3%[32*]). One possible option that Bademci offers to further improve this methodology is the utilization of spike-in baits (custom designed probes added to a predesigned TGE panel during the hybridization process) to improve coverage [24**]. This study also exemplifies some of the benefits of WES testing for hearing loss diagnosis: new gene discovery. Two of the 90 families diagnosed using their WES workflow to date segregated novel ARNSHL genes, *OTOGL* and *FAM65B* [50, 51].

CNV inclusion and methodology

CNV analysis is essential in the genetic diagnosis of hearing loss. For example, CNVs in *STRC* are a common cause of ARNSHL in Caucasian populations [52]. *STRC* encodes stereocilin, a protein essential for maintaining proper linkage of cochlear stereocillia and hearing physiology. Variants in or deletions of STRC can result in NSHL at the DFNB16

locus, but can also result in Deafness Infertility Syndrome (DIS) when *STRC* and *CATSPER2* are both deleted. In fact, Sloan-Heggen and colleagues found that *STRC* was the most common genetic cause of mild-to-moderate hearing loss, accounting for 30% of diagnoses in persons with this degree of hearing loss [32*]. Shearer et al. reported that 20% of all positive diagnoses in their cohort involved at least 1 CNV [53]. In a study of 94 patients from Germany, seven patients were diagnosed with DFNB16 NSHL or (DIS) [52]. These authors used the Omni 1-Quad v1.0 array, aCGH and qPCR to identify 9 patients with deleterious CNVs, reflecting the challenges of *STRC* CNV analysis, which is complicated by the presence of a pseudogene within the area that often undergoes non-allelic homologous recombination [53].

CNV analysis of MPS data has resulted in the identification of several novel CNVs [23*, 25, 31, 32*, 49, 54*] and has shown that many genes can harbor CNVs. Comprehensive CNV detection is as important as comprehensive SNV detection and analysis and must be included in all analytic pipelines. Two new CNV detection methods based upon differential read depth have been reported [23*, 54*], which should make CNV incorporation into TGE +MPS workflows more widespread.

Analytic pipeline and validation

Genetic testing laboratories rarely describe in detail the unique attributes of their analytic pipelines and how genetic variants are interpreted. Some labs have made their own variant scoring systems [23*] while other labs discuss results and correlate genotypes with phenotypes as part of a multidisciplinary meeting [32*]. These details can have a tremendous impact on the final report.

All TGE+MPS technologies have platform specific strengths and weaknesses for variant detection that must be established for quality control. Many labs also provide variant validation using an orthogonal technology (typically Sanger sequencing) on a newly extracted DNA sample. Although these steps may seem redundant, they are excellent checks to ensure quality and guard against errors and sample switches.

Patient specific considerations

Patient family history (including inheritance and possible parental consanguinity) and ethnicity have a major impact on the diagnostic rate. With respect to the former, Moteki et al. and Sloan-Heggen et al. showed that a positive family history for autosomal dominant NSHL or ARNSHL versus sporadic hearing loss is associated with diagnostic rates of 35% (Moteki, autosomal dominant), 35% (Moteki, AR) and 19% (Moteki, sporadic), and 50% (Sloan-Heggen, AD), 41% (Sloan-Heggen, AR) and 37% (Sloan-Heggen, sporadic), respectively [23,25].

Bademci and colleagues looked at ethnic biases. They reported an overall diagnostic rate of 56% but ethnic-specific rates of: Turkish–71% versus Iranian–24%. This variability reflects population specific coefficients of inbreeding and differences in the consanguinity rate (74% vs 35% for Turkey and Iran, for example) [49]. It may also reflect gene inclusion, as most ARNSHL genes have been identified in families from consanguinity belts in the Middle East

and India. Few studies have been done in African or African Americans, a limitation highlighted by Sloan-Heggen et al. In their analysis of 1119 probands, ethnic-specific diagnostic rates were 25% for African-Americans, 38% for European-Americans, and 72% for Middle Eastern ethnicities [32*].

Hearing loss phenotype and other physical findings suggestive of a syndrome are also important factors in the likelihood of a positive diagnosis. Of the 1119 clinical patients studied by Sloan-Heggen et al., 233 (20%) provided information indicating an additional finding (in addition to hearing loss) on physical exam. This sub-cohort had a lower-than-average diagnostic rate (27%), which varied from 0% for patients with a CNS phenotype like epilepsy to 75% for the four patients with cleft lip and palate [32*].

The most significant aspect of the hearing loss impacting diagnostic rate is laterality of hearing loss with unilateral, asymmetric and bilaterally symmetric hearing loss having diagnostic rates of 1%, 22% and 44%, respectively [32*].

Thus a patient's family history, ethnic background and physical examination can guide expectations during pretest counseling.

After testing, clinical correlation and segregation analysis

When a putative genetic diagnosis is returned, the diagnosis must be interpreted in the context of the patient. A 17-year-old given an Usher syndrome type 1 diagnosis but who did not have any motor milestone delays and has no photophobia or night blindness is incorrect due to unreasonable variance from the expected Usher 1 phenotype [55]. Similarly, a variant implicated in dominant deafness carried by an unaffected parent is not correct. Careful clinical correlation and segregation analysis are essential to proper diagnosis.

Genetic counseling is also an essential element, allowing for proper patient education and understanding of their testing results' implications for them and their families. Within regions with few genetic counselors, this educational burden will fall to the ordering clinician. As such, otolaryngologists and other ordering physicians must continue their own education in order to pursue genetic literacy.

Possible future considerations

Although hereditary hearing loss is largely monogenic and Mendelian, as comprehensive genetic testing becomes widespread, it will become possible to identify genes that alter, in both a positive and negative way, the expected phenotype. Most studies assume Mendelian inheritance, complete penetrance, and no phenocopies, however this assumption will likely not be the case, especially in large families. For example, Rehman et al. found that in 294 Pakistani families in whom they performed linkage analysis, 45 (15%) showed locus heterogeneity. They also identified a European-American family segregating 4 different hearing loss variants in 3 different genes. Presumed segregation in singleton cases may be simple, but in cases of large families, especially with multiple consanguinity loops, locus heterogeneity must be considered [56*].

Conclusion

Our knowledge of genetic hearing loss continues to grow rapidly. Providing quality testing to a patient has a significant impact on their clinical care and is recommended by the ACMG. Among the currently available tests, clinicians should select comprehensive platforms that include in the analysis pipeline discovery of both SNVs and CNVs. Genetic results should be confirmed by an orthogonal technology and must be interpreted in the context of the patient phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key points

- Comprehensive genetic testing is now possible for hearing loss.
- The ideal panel includes all genes known to cause NSHL and NSHL mimics.
- The analysis pipeline must identify both SNVs and CNVs.
- Clinical correlation is essential.
- The diagnostic rate of comprehensive genetic testing is impacted by family history, ethnic origin, type of hearing loss and phenotype.

Table 1

Clinically available comprehensive genetic testing panels

		Single Nucleotide Variations					Copy Number Variation Analysis		
Test name (Provider)	**TGE+MPS method (company)**	**Validation method**	**# genes**	**Certifications**	**TAT (days)**	**Cost (US $)**	**Available**	**Method**	**Addtl. cost**
Non-Syndromic Hearing Loss (University of Minnesota Physicians Outreach Laboratory with Fairview Diagnostics)	Method not specified (not specified)	NA	**NA**	CLIA, CAP	56	NA		NA	
OtoSeq (Cincinnati Children's Hospital Medical Center)	Microdroplet PCR (RainDance Technologies)	NA	**23**	CLIA, CAP	90	Var.	Yes	aCGH	Var.
Nonsyndromic Hearing Loss and Deafness NextGen Sequencing (NGS) Panel (Prevention Genetics)	DNA baits (not specified)	Sanger Sequencing	**49**	CLIA, CAP	45	1990	Yes	aCGH	1670
Expanded Hearing Loss Panel (ARUP Laboratories)	Method not specified (not specified)	NA	**56**	CLIA, CAP	70–84	NA	Yes	aCGH	Yes
NGS Hearing Loss Panel (Greenwood Genetic Center)	SureSelectXT Clinical Research Exome and selective filtration (Agilent Technologies)	Sanger Sequencing	**84**	CLIA, CAP	56–70	3500	No		
OtoGenome (Harvard Medical School and Partners Healthcare)	SureSelect RNA baits (Agilent Technologies)	Sanger Sequencing	**87**	CLIA	56–84	3800	Yes	VisCap (MPS data) & ddPCR	No
Hearing Loss: Sequencing Panel (Emory Genetics Laboratory)	In-solution hybridization (not specified)	NA	**91**	CLIA, CAP	84	NA	Yes	aCGH	Yes
Hearing Loss NGS Panel (Fulgent Diagnostics)	DNA baits (Illumina technologies)	Sanger Sequencing	**103**	CLIA, CAP	28–42	1450	Yes	MPS data (confirmed)	500
Hearing Loss Panel (Knight Molecular Diagnostic)	TruSight One (Illumina Technologies)	NA	**121**	CLIA, CAP	56	2624	Yes	aCGH	Yes

		Single Nucleotide Variations					Copy Number Variation Analysis		
Test name (Provider)	**TGE+MPS method (company)**	**Validation method**	**# genes**	**Certifications**	**TAT (days)**	**Cost (US $)**	**Available**	**Method**	**Addtl. cost**
Syndromic and non-syndromic deafness (NGS panel for 127 genes) (CGC Genetics USA)	SureSelect RNA baits (Agilent Technologies)	Sanger Sequencing	**127**	CLIA	60	1950–2500	Yes, limited	MLPA	Yes
Deafness Gene Panel (Otogenetics)	TotSeq (Otogenetics)	Sanger Sequencing	**129**	CLIA	42–84	NA		NA	
OtoSCOPE (v7) (University of Iowa Hospitals and Clinics-Molecular Otolaryngology and Renal Research Laboratories)	SureSelect RNA baits (Agilent Technologies)	Sanger Sequencing	**133**	CLIA	90–120	1500	Yes	MPS data	No

NA: information not available to the authors. For further information or questions, please contact the ordering lab.