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Massively Parallel Sequencing for Genetic Diagnosis of Hearing Loss: The New Standard of Care

A. Eliot Shearer, M.D., Ph.D.[a] and **Richard J.H. Smith, M.D.**[a,b,c,^]

[a]Department of Otolaryngology—Head and Neck Surgery, University of Iowa Carver College of Medicine, Iowa City, Iowa, USA

[b]Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, Iowa, USA

[c]Department of Molecular Physiology & Biophysics, University of Iowa College of Medicine, Iowa City, Iowa, USA

Abstract

Objective—To evaluate the use of new genetic sequencing techniques for comprehensive genetic testing for hearing loss.

Data Sources—Articles were identified from PubMed and Google Scholar databases using pertinent search terms.

Review Methods—Literature search identified 30 studies as candidates that met search criteria. Three studies were excluded and eight studies were found to be case reports. 20 studies were included for review analysis including seven studies that evaluated controls and 16 studies that evaluated patients with unknown causes of hearing loss; three studies evaluated both controls and patients.

Conclusions—In the 20 studies included in review analysis, 426 control samples and 603 patients with unknown causes of hearing loss underwent comprehensive genetic diagnosis for hearing loss using massively parallel sequencing. Control analysis showed a sensitivity and specificity > 99%, sufficient for clinical use of these tests. The overall diagnostic rate was 41% (range 10% to 83%) and varied based on several factors including inheritance and pre-screening prior to comprehensive testing. There were significant differences in platforms available in regards to number and type of genes included and whether copy number variations were examined. Based on these results, comprehensive genetic testing should form the cornerstone of a tiered approach to clinical evaluation of patients with hearing loss along with history, physical exam, and audiometry and can determine further testing that may be required, if any.

Implications for Practice—Comprehensive genetic testing has become the new standard of care for genetic testing for patients with sensorineural hearing loss.

[^]To whom correspondence should be addressed: Richard J.H. Smith, MD, Department of Otolaryngology—Head and Neck Surgery, 200 Hawkins Drive, Iowa City, IA 52242, Office Phone: 319-356-3612, richard-smith@uiowa.edu.

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Conflicts of Interest Both authors are members of the non-profit fee-for-service Molecular Otolaryngology & Renal Research Labs at the University of Iowa, which offers a comprehensive genetic test for deafness using massively parallel sequencing.

Keywords

Deafness; hearing loss; genetic testing; genomics

Introduction

Hearing loss is the most common sensory deficit in humans. It affects 1 in 500 newborns and over 360 million people worldwide. In developed countries the majority of congenital sensorineural hearing loss (SNHL) is non-syndromic (NSHL, not associated with any other abnormalities) and genetic. Unlike some other well-known genetic disorders caused by a single mutation (cystic fibrosis) or mutations in a single gene (Duchenne muscular dystrophy) in the majority of cases, there are more than 80 genes and more than a thousand reported deafness-causing mutations. This extreme genetic heterogeneity makes genetic diagnosis for NSHL exceedingly difficult.

This difficulty in diagnosis is crucial to overcome as a genetic diagnosis provides important prognostic and genetic heritability information to patients, is helpful in excluding syndromic causes of hearing loss, and can prevent other unnecessary and costly testing. As new technological advances in genetic sequencing have emerged, clinical genetic diagnosis for hearing loss has evolved from single mutation testing to methods available today that allow comprehensive genetic testing whereby hundreds of genes are sequenced simultaneously.

From a practical standpoint DNA sequencing requires two steps: enrichment of the genetic region of interest and sequencing. Genetic testing has traditionally been performed using Sanger sequencing, first developed in 1977 [1]. Sanger sequencing relies on polymerase chain reaction (PCR) to isolate individual regions of the genome (typically exons), which are then subjected to sequencing. This method has an extremely high sensitivity and specificity and ushered gene sequencing in to a clinical setting. However Sanger sequencing is hampered by low throughput and high cost. Typically, all exons of a single gene may sequenced with this method at a cost in clinical laboratory ranging from $1,000–$3,000 per gene with a turn around time of about 3 months per gene. Comprehensive testing for genetically heterogeneous disorders such as NSHL is infeasible using Sanger sequencing due to cost and time constraints.

Massively parallel sequencing (MPS) was developed in the wake of the completion of the human genome project to improve throughput and decrease costs associated with DNA sequencing. MPS in general relies on targeted genomic enrichment (TGE) for simultaneous isolation of hundreds or thousands of genomic regions prior to high-throughput sequencing. A detailed description of MPS technology is outside the scope of this review but can be found elsewhere [2–4]. Sequenced genetic regions can include only exons or gene regions of interest (a targeted disease specific gene panel) or all exons of all genes in the genome (exome sequencing).

The first studies successfully demonstrating MPS for DNA sequencing were published in 2005[5], followed by many studies demonstrating the high throughput and accurate nature of this method for use in a variety of genetic disorders (reviewed in [6]). A study published in

2010 in which the *BRCA* gene region was sequenced was the first to demonstrate effective diagnosis of a human genetic disease with TGE and MPS[7]. That same year the first study showing the effectiveness of this method for diagnosis of hearing loss was published[8]. Since then there have been a large number of studies published using this methodology for genetic diagnosis of deafness.

The goal of this review is to summarize the findings from the studies in the past five years using MPS as a method for comprehensive diagnosis of deafness. These studies evaluate the use of these new technologies for clinical diagnostics by examining standard clinical testing parameters using controls (including sensitivity and specificity of the method) as well as the diagnostic ability of this new type of test in patients affected by hearing loss. Our goal in this review is to provide context for clinicians that will be ordering and interpreting results from these newly developed tests.

Methods

We performed a literature search using PubMed and Google Scholar databases as of February 2015. Search criteria included several keywords used in varying combinations: “deafness”, “hearing loss”, “massively parallel sequencing”, and “next-generation sequencing”. Studies were excluded if the study used pooled DNA sequencing or linkage analysis as these techniques would not be routinely used in clinical diagnostics.

Discussion

Studies identified for review

All 30 studies identified through the literature search are included in Table 1. We identified 27 studies that met our criteria for inclusion and three that were excluded. Exclusions were due to use of pooled DNA samples in one case[9] and linkage analysis used in two cases[10,11]. Eight of the 27 studies were case reports that primarily highlight the unique ability of comprehensive genetic testing to determine complex genetic causes of hearing loss. These eight studies were not part of analysis for review except for one study[12], which included 10 control samples in addition to the case report.

There were seven studies that evaluated MPS with the use of controls. There were 16 studies that used MPS to evaluate patients with unknown causes of hearing loss with three studies including both controls and patients with unknown causes of hearing loss (Table 1).

Studies evaluating MPS platforms with control individuals

Prior to using a new technology for a clinical diagnostic test, the new test should be evaluated for sensitivity and specificity using control samples. Although Sanger sequencing has a high cost and low throughput, it has excellent specificity and sensitivity for individually targeted regions. Any new genetic screening technology should be compared against this current gold standard.

We identified seven studies evaluating MPS technologies for clinical diagnosis of hearing loss using 425 control individuals with previously identified causative genetic mutations and

one study [13] which used a publically available HapMap DNA sample for formal sensitivity and specificity analysis (Table 2). These studies used two methods for genomic region isolation: targeted genomics enrichment and microdroplet PCR. Two sequencing methods were used in these seven studies with Illumina sequencing being the most common (7 of 8, 88%), and one study using Ion Torrent sequencing.

In the largest study we identified, examining 384 controls, MPS detected 159/174 control mutations for an overall true positive diagnostic rate of 91.4%[14]. The 15 control mutations that were missed were located at mutation sites that were not included on the targeted enrichment platform, underscoring the importance of platform design. In the remaining five studies, 100% of the positive control mutations were identified in 41 samples using MPS.

Three studies included formal sensitivity and specificity analysis including two studies which compared MPS to gold-standard Sanger sequencing[8,15] and one study in which MPS was compared to a reference human genome sequence in a publically available HapMap sample[13]. Sensitivity and specificity were both >99% in all three studies when evaluating a total of more than 1,500 genotype calls. These data indicate that MPS is suitable for clinical genetic diagnosis of hearing loss.

Studies evaluating patients with unknown causes of hearing loss

We identified 16 studies in which MPS was used for genetic testing of individuals with unknown causes of hearing loss (Table 3). In total there were 603 individuals tested. The number of individuals per study varied from 6 to 125. The majority (88%, 14/16) of these studies used targeted genomic enrichment prior to sequencing while one study used microdroplet PCR and one study used whole exome sequencing. Illumina sequencing was used for all of the studies.

The studies varied considerably in the number of hearing loss genes targeted for sequencing from 15 to 246 genes. The current number of genes identified as harboring mutations that cause human NSHL is 84 (http://www.hereditaryhearingloss.org). There are several reasons why the number of genes varies between studies including: 1) deafness genes are still being discovered and so the number increases over time, 2) in some cases authors include genes that cause syndromic forms of hearing loss (i.e. Usher syndrome or Pendred syndrome), and 3) some authors include genes that cause hearing loss in mice or have been identified as excellent candidate genes for human deafness in previous studies but have not yet been implicated in human deafness. When ordering an MPS test for clinical diagnosis of NSHL it is important to understand which genes are included and why as this information is crucial in determining the meaning of a “negative” test.

In the majority of studies (81%, 13/16) individuals with unknown causes of hearing loss were pre-screened for common deafness mutations prior to undergoing comprehensive genetic testing. This likely adequately reflects patients who may present with a request for comprehensive genetic testing for deafness after having previously been tested negative for mutations in the most common gene(s) (i.e. *GJB2*).

Diagnostic rate of MPS

Across the 16 studies that included 603 individuals with unknown causes of hearing loss tested with massively parallel sequencing, the diagnostic rate overall averaged 41% and ranged from 10% to 83% (Table 3). The study with the lowest diagnostic rate, Eppsteiner et al. 2012, focused on adults with hearing loss and therefore may have had an ascertainment bias towards individuals with environmental or noise-induced non-genetic hearing loss[16]. Gu et al. 2014, found a diagnostic rate of 13%, however the patients were strictly pre-screened and were all sporadic patients with no family history of hearing loss[17]. Shearer et al. 2010 had the highest diagnostic rate but also the smallest sample size (n=6) and so there may have been ascertainment bias[8].

Inheritance mode of hearing loss was specified in 69% of studies (11/16). Diagnostic rate was lower for individuals with autosomal recessive or sporadic inheritance (40%) when compared with the 65% diagnostic rate for individuals with autosomal dominant inheritance.

Analysis for point mutations and small deletions is routine for genetic sequencing. However, only 31% of studies (5/16) screened for large copy number variations. Copy number variations are increasingly understood to be a common cause of genetic hearing loss, accounting for between 13% and 19% of all causative mutations in two studies[18,19]. Another study identified copy number variations as commonly present in hearing loss genes[20]. Others have gone so far as to advocate copy number variation analysis as a requirement for all patients undergoing genetic testing for hearing loss due to the large carrier frequency of copy number variations in the *STRC* gene region[21].

Case Reports and Exome Sequencing

We also identified eight reports that detailed cases in which comprehensive genetic testing was essential for diagnosis or highlighted the unique features of MPS (Table 1). Five of these reports used whole exome sequencing (WES). This is a method of targeted genomic enrichment whereby every exon of every gene in the human genome is isolated and enriched prior to sequencing. WES has the advantage of casting a broader net for diagnosis but comes with an increased cost of reagents and analysis. In addition, incidentally identified variants in genes not involved in hearing loss will be uncovered.

One study identified a syndromic form of hearing loss (Usher syndrome) in a patient with apparent non-syndromic hearing loss using a deafness specific panel[12]. Two of the case reports used comprehensive genetic testing for diagnosis of families with and found both non-syndromic and syndromic forms of hearing loss segregating simultaneously[22,23]. Two studies identified families with three forms of hearing loss segregating simultaneously[23,24]. And one study identified a possibly life threatening disorder, Long QT syndrome caused by a mutation in *KCNQ1*, with exome sequencing, in a patient with what appeared to be non-syndromic hearing loss [25]. These cases underscore the versatility of comprehensive genetic diagnosis for complex familial cases and the role of WES for complicated pedigrees.

Implications for Practice

Since the first use of MPS for genetic diagnosis of hearing loss five years ago, there have been 28 other studies published using this new methodology. In total, 7 studies evaluated 427 control patients to assess this methodology as a clinical diagnostic test, including formal sensitivity and specificity analysis in three studies. There were 16 studies evaluating the effectiveness of MPS technologies for diagnosis of NSHL in 603 individuals with unknown causes of hearing loss.

The data from the 20 studies reviewed here indicate that comprehensive genetic diagnosis using massively parallel sequencing is suitable for clinical use. It provides a better overall diagnostic rate on varying ethnicities (41%) than single gene testing, which must be tailored to the phenotype and population being studied and for single gene testing. For example, mutations in the gene *GJB2* are the cause of between 15–40% of autosomal recessive NSHL in Caucasian individuals [26] but mutations in this same gene very rarely cause genetic hearing loss in other populations [27]. This issue led to heated debate over the appropriate sequentially ordered single-gene test for a specific population and type of hearing loss[28]. Comprehensive deafness-specific testing has allowed clinical testing to move beyond that debate.

There are four comprehensive genetic tests for hearing loss currently available in the United States (Table 4). Costs have decreased such that now the cost for comprehensive genetic testing approach or are at the same level as single gene testing. Comprehensive genetic testing has quickly become the standard of care for genetic diagnosis of sensorineural hearing loss.

This review also sheds light on several current issues regarding clinical comprehensive genetic testing for deafness that have yet to be resolved. A clinician ordering one of these tests should be aware of these controversies. First, the number and type of genes included in the platform can vary considerably. As shown in this review there can be considerable variation in the number of genes included on a “comprehensive” test, ranging in our review from 34–246 different genes (Table 3) and from 23–129 in currently available clinical genetic tests (Table 4). As previously described, the genes included on the platform varies based on whether only non-syndromic hearing loss genes are included, whether syndromic deafness genes are included (and which syndromes), and whether genes that are predicted to cause deafness in humans (either via animal studies or other analysis) are included.

It may seem that more is always more, but when performing genetic testing, incidental findings are of considerable concern[29,30]. Patients may not wish to know carrier status for specific diseases or risk alleles associated with diseases unrelated to the condition for which they obtained the test. Using a more targeted test reduces the risk of incidental findings. However, the benefit of including syndromic genes is that this may provide diagnoses in patients for whom a mutation in one of these genes is not suspected. For example, in one case report, a patient with presumed non-syndromic hearing loss was diagnosed with long QT syndrome, which can potentially be fatal [25]. There have been several reports of Usher syndrome diagnosis in patients with apparent non-syndromic hearing loss[12,18]. This ability

to provide a diagnosis must be weight against incidental genetic findings. Like all incidental findings in medicine, genetic findings lead to an increase burden of referrals and other testing that the patient may not have wished for. Thus, while exome sequencing is available, it carries an increased chance of incidental findings, as well as increased cost and increased difficulty with analysis. One study comparing a disease-focused panel versus exome sequencing for inherited eye diseases found improved accuracy and performance of the disease-specific panel, a finding that also applies to panels for hearing loss [31]. For these reasons, disease-focused genetic tests have become the standard when evaluating hearing loss[6]. As illustrated by the case reports presented, exome sequencing may be valuable for more complex indications and when a deafness-specific panel has failed to determine a cause.

Finally, when considering a comprehensive genetic test, the type of mutations evaluated must be considered. All platforms include analysis of point mutations and small deletions, but large insertions or deletions are crucial for any comprehensive genetic test as these genetic alterations have been shown to be responsible for 13 or 19% of all deafness in two studies [18,32]. Other groups have also advocated copy number variation analysis in all cases [20,21].

MPS has now become well-established as a clinical diagnostic tool for deafness and other genetic disorders and have become a “cornerstone” of clinical genetic testing [6]. The American College of Medical Genetics has developed laboratory standards for diagnostic laboratories to adhere to when performing diagnostic MPS tests and clinicians should ensure that these standards are used by the laboratory performing the test they have ordered [33]. And as final evidence that MPS testing is now integral to effective diagnosis of deafness, the newest guideline from the American College of Medical Genetics for the evaluation of NSHL includes MPS testing as part of the standard algorithm for diagnosis[34].

Comprehensive genetic testing using MPS should now form the standard of care for genetic evaluation of patients with hearing loss. Diagnostic rates will continue to improve as new causes of hearing loss are discovered. As comprehensive hearing loss panels become more widely used, more patients will be able to obtain a genetic diagnosis, which will provide prognostic and heritability information to patients. Having a genetic diagnosis may also guide decisions on cochlear implantation[16,35] and is the first step in designing tailor-made genetic therapies[36].

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Table 1

Studies evaluated in this review, ordered by year.

Study (reference)	Exclusions	Case Report	Evaluated Controls	Evaluated Unknowns
Shearer et al. 2010 [8]			yes	yes
Brownstein et al. 2011 [37]				yes
Baek et al. 2012 [38]				yes
De Keleulenaer et al. 2012 [11]	Linkage analysis			
Diaz-Horta et al. 2012 [39]				yes
Eppsteiner et al. 2012 [16]				yes
Tang et al. 2012 [40]			yes	
Wei et al. 2012 [12]		yes	yes	
Choi et al. 2013 [41]				yes
Gao et al. 2013 [42]		yes		
Miyagawa et al. 2013 [9]	Pooled analysis			
Mutai et al. 2013 [43]				yes
Shahzad et al. 2013[10]	Linkage analysis			
Schrauwen et al. 2013[13]			yes	yes
Shearer et al. 2013 [18]				yes
Sivakumaran et al. 2013[15]			yes	
Wu et al. 2013[44]				yes
Yang et al. 2013[45]				yes
Behar et al. 2014[22]		yes		
Cheng et al. 2014[46]		yes		
Gu et al. 2014[17]				yes
Haraksingh et al. 2014[47]		yes		
Ji et al. 2014[20]				yes
Lu et al. 2014[23]		yes		
Park et al. 2014[35]				yes
Qing et al. 2014[24]		yes		
Tekin et al. 2014[25]		yes		
Wei et al. 2014[48]				yes
Vona et al. 2014[49]			yes	yes
Nishio et al. 2015[14]			yes	yes

Table 2

Studies evaluating massively parallel sequencing for clinical diagnostics for genetic hearing loss using controls, ordered by year.

Study	Enrichment Method	Sequencing Method	Genes Sequenced	Samples	Positive Ctrl Dx
Shearer et al. 2010[*]	TGE	Illumina	54	4	100%
Tang et al. 2012	TGE	Illumina	5	10	100%
Wei et al. 2012	TGE	Illumina	69	10	100%
Schrauwen et al. 2013[*#]	MicroPCR	Illumina	34	1	-
Sivakumaran et al. 2013[*]	MicroPCR	Illumina	24	8	100%
Vona et al. 2014	TGE	Illumina	80 or 129	9	100%
Nishio et al. 2015	MicroPCR	IonTorrent	63	384	91.4%

Abbreviations: MicroPCR, microdroplet PCR; TGE, targeted genomic enrichment

[*] Study includes formal sensitivity and specificity analysis.

[#] Study used a human reference genome HapMap sample for control analysis, see text for details.

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Table 3

Studies evaluating massively parallel sequencing for clinical diagnostics for genetic hearing loss with individuals with unknown causes of hearing loss, ordered by year.

Study	Enrichment Method	Sequencing Method	n Genes Sequenced	Ethnicity	CNV analysis	Prescreened	n Samples	Diagnostic Rate	AR/Sporadic Diagnostic Rate*	AD Diagnostic Rate*
Shearer et al. 2010	TGE	Illumina	54	Caucasian	no	yes	6	83%	100%	75%
Brownstein et al. 2011	TGE	Illumina	246	Israeli Jewish and Palestinian Arab	no	yes	11	55%	40%	100%
Baek et al. 2012	TGE	Illumina	80	Korean	no	yes	8	63%	-	63%
Diaz-Horta et al. 2012	WES	Illumina	exome	Turkey/Iran	no	no	20	60%	60%	-
Eppsteiner et al. 2012	TGE	Illumina	59	Mixed	no	no	29	10%	-	-
Choi et al. 2013	TGE	Illumina	80	Korean	no	yes	20	60%	57%	69%
Mutai et al. 2013	TGE	Illumina	84	Japanese	no	yes	15	47%	-	-
Schrauwen et al. 2013	MicroPCR	Illumina	34	European	no	yes	24	38%	38%	-
Shearer et al. 2013	TGE	Illumina	54, 59, or 66	Mixed	yes	yes	100	42%	46%	31%
Wu et al. 2013	TGE	Illumina	80	Chinese	no	yes	12	33%	0 %	60%
Yang et al. 2013	TGE	Illumina	79	Chinese	no	yes	125	26%	25%	57%
Gu et al. 2014	TGE	Illumina	131	Chinese	yes	yes	63	13%	13%	-
Ji et al. 2014	TGE	Illumina	80	Chinese	yes	no	79	27%*	27%	-
Park et al. 2014	TGE	Illumina	204	Korean	yes	yes	45	24%	-	-
Vona et al. 2014	TGE	Illumina	80 or 129	European	yes	yes	23	52%	-	-
Wei et al. 2014	TGE	Illumina	104	Chinese	no	yes	23	30%	-	-

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; TGE, targeted genomic enrichment, WES, whole exome sequencing.

*In this study the diagnostic rate varied from 27–37% depending on criteria used; 27% was used for analysis.

Table 4

Currently available comprehensive genetic tests for deafness in the United States, ordered by test name.

Test	Laboratory	Method	CNV	n Genes	TAT	Cost
OtoGenetics Deafness Test	OtoGenetics Corporation	TGE+MPS	no	129	5–6 wks	$596*
OtoGenome	Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine	TGE+MPS	yes	89	6–8 wks	$3,800
OtoSCOPE	University of Iowa Molecular Otolaryngology & Renal Research Labs	TGE+MPS	yes	116	12 wks	$1,500
OtoSeq	Cincinnati Children's Hospital Medical Center, Molecular Genetics Laboratory	TGE+MPS	no	23	12–13 wks	$3,625

Abbreviations: TAT, turn-around-time; CNV, copy number variation; TGE, targeted genomic enrichment; MPS, massively parallel sequencing.

*Cost includes test, basic bioinformatics analysis, and DNA extraction fee.