Development of in-house genetic screening for pediatric hearing loss

Karl W. Doerfer MD1 | Tara Sander PhD2 | Girija G. Konduri MD3 | David R. Friedland MD, PhD1 | Joseph E. Kerschner MD1 | Christina L. Runge PhD1

1Medical College of Wisconsin, Department of Otolaryngology & Communication Sciences, Milwaukee, Wisconsin
2Charlotte Lozier Institute
3Medical College of Wisconsin, Department of Neonatology, Milwaukee, Wisconsin

Abstract

Objectives: To evaluate the efficiency of in-house genetic testing for mutations causing the most common types of inherited, nonsyndromic, sensorineural hearing loss (SNHL).

Methods: Retrospective cohort study of 200 patients at a single, pediatric medical center with suspected or confirmed hearing loss who underwent either send-out vs in-house genetic testing for mutations in GJB2/GJB6, SLC26A4, and MTRNR1. Primary outcome measure was the difference in mean turnaround time for send-out vs in-house genetic testing. Additional outcomes included associations between audiometric findings and genetic test results.

Results: One hundred four send-out tests were performed between October 2010 and June 2014, and 100 in-house tests were performed between November 2014 and November 2016. The mean turnaround time for send-out testing was 53.7 days. The mean turnaround time for in-house testing was 18.9 days. This difference was statistically significant (P < .001). The largest component of turnaround time was the amount of time elapsed between receipt of specimen in the lab and final test result. These intervals were 47.0 and 18.3 days for send-out and in-house tests, respectively. Notably, the longest turnaround time for in-house testing (43 days) was less than the average turnaround time for send-out testing. In addition, we identified two simple audiometric parameters (ie, bilateral newborn hearing screen referral and audiometry showing symmetric SNHL) that may increase diagnostic yield of genetic testing.

Conclusions: The development of in-house genetic testing programs for inherited SNHL can significantly reduce testing turnaround times. Newborn hearing screening and audiometry results can help clinicians identify patients most likely to benefit from genetic testing.

Level of Evidence: IV.

KEYWORDS
congenital hearing loss, genetic testing, sensorineural hearing loss

This research was presented as a poster at the Triological Society Combined Sections Meeting in Scottsdale, Arizona, USA January 18-20, 2018.
1 | INTRODUCTION

Sensorineural hearing loss (SNHL) affects approximately 1.86/1000 live births, and prevalence is higher in certain settings, such as neonatal intensive care units, where rates are approximately 2-4/1000.1,2 The prevalence of SNHL rises throughout childhood, with 2.7/1000 affected by age 5 and 3.5/1000 affected by adolescence.1,3 Failure to treat SNHL can have deleterious effects on a child’s development, quality of life, and eventual socioeconomic standing. Furthermore, the window of opportunity to intervene is narrow: children with congenital SNHL diagnosed after 6 months of age are at high risk for lagging behind peers in terms of speech and language development.4-7

Since the 1990s, newborn hearing screening (NBHS) programs have allowed for diagnosis and intervention of congenital SNHL within the first months of life.8 Although NBHS programs play a critical role in early identification and intervention for hearing loss, current screening protocols have limitations, including: (a) the inability to identify children with mild SNHL whose hearing levels fall beneath the thresholds of screening tests; and (b) missing the identification of children at risk for late-onset or progressive SNHL, some of which have genetic causes. Often, these children are not identified until years later through school-based screening or formal audiologic assessment prompted by concerned caregivers.9,10

Approximately 70% of congenital SNHL has a genetic basis. By the age of four, congenital SNHL accounts for 54% of cases; however, a substantial proportion of later-onset SNHL is related to inherited vulnerabilities.5 The most commonly implicated genes (GJB2/GJB6), which account for approximately 35% of congenital deafness, may possess mutations that lead to late onset, progressive loss during early childhood.10,11 Recent studies have shown that up to 56% of patients with these mutations may pass initial NBHS.12 Additionally, some genetic mutations raise susceptibility for acquired hearing loss through toxic and environmental exposures. For example, patients with mutations in SLC26A4, the gene responsible for Pendred Syndrome and the nonsyndromic hearing loss phenotype DFNB4, are at risk for SNHL from minor head trauma. Similarly, mutations in the mitochondrial gene MTRNR1 can lead to hearing loss through exposure to aminoglycosides.13,14

In recent years, genetic testing has played an increasingly important role in the diagnosis of neonatal and pediatric hearing loss. Historically, laboratory studies for identifying inherited SNHL included tests such as electrolyte analysis, urinalysis, thyroid function, antinuclear antibody, rheumatoid factor, CBC, electrocardiogram, and others. However, research has shown that these tests have a low diagnostic yield (0%-2%) and they provide minimal information about underlying mutations.15 In contrast, genetic testing has greater sensitivity and specificity, and results can provide detailed information to optimize patient care.15 For example, GJB2 mutations causing significant hearing loss, which are well-characterized with genetic testing, produce no abnormal routine lab or imaging findings. Yet, multiple studies have shown these individuals to be highly successful cochlear implant recipients.16-18 Genetic testing results for these patients is highly useful in combination with other diagnostic findings to expedite intervention, including cochlear implant surgery, and in providing valuable prognostic information to parents and providers. Similarly, early identification of mutations in SLC26A4 and MTRNR1 may help implement preventative measures that would minimize the risk of progressive loss caused by trauma or ototoxic exposure.

NBHS combined with genetic testing holds significant promise for improving diagnosis of pediatric SNHL. However, high cost and long turnaround times have limited the feasibility of broad adoption. More recently, genetic testing costs have fallen with advances in sequencing technology, as well as with the development of standardized, epidemiologically based panels to target genes of interest. As testing becomes more widespread, costs are expected to decline further.19 Lengthy turnaround times remain a potential limit to the utility of genetic testing. Currently, high-volume genetic testing is primarily performed at a limited number of centers in the United States, and test results may not be available for several months, potentially delaying diagnosis and intervention.20

Current screening for inherited SNHL has several limitations, including inability to detect mild SNHL and later onset loss due to genetic susceptibility. Furthermore, traditional diagnostic laboratory testing for patients with confirmed SNHL provides little information to determine prognosis and guide management. Genetic testing can address many of these limitations; however, the long turnaround time associated with such testing continues to limit its utility. In response, our institution developed an in-house genetic testing panel for the most common genetic causes of SNHL in the United States. This study evaluated whether in-house testing can significantly reduce turnaround times, thus removing a significant barrier to broader adoption. In addition, this study evaluated whether certain NBHS and audiometry results can be used to increase the diagnostic yield of genetic testing.

2 | MATERIALS AND METHODS

This retrospective chart review examined all pediatric patients undergoing genetic testing for SNHL between October 2010 and November 2016. Two hundred patients were identified. In-house genetic tests were developed and performed in a CLIA and CAP-accredited laboratory. The exons and immediately adjacent intronic regions of GJB2, including the 5’ prime UTR known to harbor a pathogenic mutation, were PCR amplified and analyzed by Sanger sequencing. Four MTRNR1 gene mutations (827A>G, 961delT+C(n) ins, 1494C>T, and 1555A>G) were analyzed by Sanger sequencing. Real-time PCR Taqmam assays were used to perform SLC26A4 mutation analysis for the most common mutations associated with Pendred syndrome and nonsyndromic enlarged vestibular aqueduct (c.707T>C and c.1246A>C). Taqmam copy number assays in a quantitative real-time PCR-based genotyping platform were designed to detect deletions encompassing the GJB6 gene (309 kb del [GJB6-D13S1830] and 232 kb del[GJB6-D13S1854]). Send-out testing was done for GJB2 and GJB6 mutations, using the same methods as were used in-house. Patient testing was ordered from a variety of settings, including outpatient clinics (otolaryngology, genetics and general pediatrics) as well as the neonatal intensive care unit. In addition, this study evaluated whether certain NBHS and audiometry results can be used to increase the diagnostic yield of genetic testing.
From October 2010 to October 2013, only send-out testing was available. As of November 2013, in-house genetic testing became available, and as of July 2014 all testing was done exclusively in-house. In all, 104 send-out tests were performed, and 100 in-house tests were performed (some patients had both tests performed during the time of transition).

To measure time associated with testing, the following time points were captured: date of specimen collection, date of sample acquisition by the lab, result date, and date results were clinically available. To characterize the study cohort, we reviewed medical records for basic demographic data, audiologic assessments, and interventions for treating SNHL. Audiologic assessments were obtained from the most complete testing done closest to the time of genetic testing. These included NBHS, behavioral testing (visual reinforcement audiometry), pure tone audiometry, DPOAEs, and diagnostic auditory brain response (ABR). Due to the diversity of audiologic data, type and degree of hearing loss were defined by the highest reported threshold in the worst-hearing ear. Hearing loss was classified per convention with respect to type (ie, sensorineural, conductive, mixed) and severity (ie, mild, moderate, moderately severe, severe, profound). Laterality of hearing loss was also considered and classified as symmetric, asymmetric, unilateral (ie, one ear with normal thresholds), or unknown.

The primary outcome analysis was difference in mean turnaround time for send-out vs in-house testing. Turnaround time was defined as the number of days elapsed between specimen collection and result availability. Data were analyzed for statistical significance using Student's t test. Secondary outcome measures included: (a) genetic testing results vs NBHS results; (b) genetic testing results vs hearing loss laterality; and (c) genetic testing results vs hearing loss severity. These results were analyzed using chi-square and Fischer's exact tests.

### RESULTS

#### 3.1 Turnaround times, costs, and genetic testing results

One hundred four send-out tests were performed between October 2010 and June 2014, and 100 in-house tests were performed between November 2014 and November 2016 (Figure 1).
Average send-out testing cost was $3180, whereas average in-house testing was $2238, a difference of $942. In most cases, insurance covered the cost of testing. Four patients had both send-out and in-house testing ordered. Turnaround time data for these duplicated tests were included in the final analysis. The mean turnaround time for send-out testing was 53.7 days (median 49, range 17-142). The mean turnaround time for in-house testing was 18.9 days (median 18, range 7-43). This difference was statistically significant ($P < .001$; Figure 2). The largest component of turnaround time was the amount of time elapsed between receipt of specimen in the lab and final test result. The mean intervals for these time frames were 47.0 days (range 10-140 days) and
18.3 days (range 7-43 days) for send-out and in-house tests, respectively. The difference between these values was statistically significant \( (P < .001) \). Similar differences were seen in time from collection to arrival in the lab (send-out 2.1 days, in-house 0.6 days) and time from final test results to results being available to our institution (send-out: 4.6 days, in-house 0.0 days). However, these intervals were minor contributors to the overall turnaround time. Notably, the longest turnaround time for in-house testing (43 days) was less than the average turnaround time for send-out testing. To evaluate whether turnaround time for send-out testing declined over time due to increased testing efficiency, we compared the average turnaround time during the first and last 12 months of send-out testing. A decline was noted (58.8 vs 48.2 days) but this difference was not significant \( (P = .084) \).

### 3.2 Patient characteristics

Patients undergoing genetic testing included 90 females and 110 males, with ages ranging from 1 day to 18 years. In all, 32% of

### Table 1  List of mutations categorized by genetic condition and including testing status (positive, negative, uncertain)

| Mutations                          | (+) Test | (−) Test | Uncertain result | Grand total |
|------------------------------------|----------|----------|------------------|-------------|
| DFNB1                              | 19       |          |                  | 19          |
| 1) c.35delG, 2) c.35delG (GJB2)     | 9        |          |                  | 9           |
| 1) c.109G>A, 2) c.109G>A (GJB2)     | 2        |          |                  | 2           |
| 1) c.35delG, 2) c.109G>A, 3) c.35delG (GJB2) | 2 | | | 2 |
| 1) c.269T>C (GJB2)                 | 2        |          |                  | 1           |
| 1) c.35delG, 2) c.229T>C (GJB2)     | 1        |          |                  | 1           |
| 1) c.35delG, 2) c.139G>A (GJB2)     | 1        |          |                  | 1           |
| 1) c.35delG, 2) c.71G>A (GJB2)      | 1        |          |                  | 1           |
| 1) c.35delG, 2) c.358_360delGAG, 3) c.380G>T (GJB2) | 1 | | | 1 |
| 1) c.3170G>A, 2) c.269C>T (GJB2)    | 1        |          |                  | 1           |
| Likely DFNB1                        | 4        |          |                  | 4           |
| 1) c.269T>C, 2) c.167delT (GJB2)    | 1        |          |                  | 1           |
| 1) c.35delG, 2) c.101T>C (GJB2)     | 1        |          |                  | 1           |
| 1) c.109G>A, 2) c.617A>G (GJB2), 3) m.827A>G (MT-RNR1) | 1 | | | 1 |
| 1) c.35delG, 2) c.267dupC (GJB2)    | 1        |          |                  | 1           |
| DFNA3/palmoplantar keratoderma      | 1        |          |                  | 1           |
| 1) c.223C>T, 2) Normal allele (GJB2) | 1 | | | 1 |
| Benign polymorphism (GJB2)         | 17       |          |                  | 17          |
| Benign polymorphism (GJB2)         | 17       |          |                  | 17          |
| Carrier                            | 13       |          |                  | 13          |
| c.35delG (GJB2)                    | 7        |          |                  | 7           |
| c.617A>G (GJB2)                    | 1        |          |                  | 1           |
| c.416G>A (GJB2)                    | 1        |          |                  | 1           |
| c.269T>C (GJB2)                    | 1        |          |                  | 1           |
| c.133G>A (GJB2)                    | 1        |          |                  | 1           |
| c.109G>A (GJB2)                    | 1        |          |                  | 1           |
| c.101T>C (GJB2)                    | 1        |          |                  | 1           |
| Heterozygous mutation of unknown significance | 1   | | | 1 |
| c.241C>G (GJB2), m.827A>G (MT-RNR1) | 1 | | | 1 |
| Grand total                        | 24       | 30       | 1                | 55          |
patients underwent genetic testing before 1 year of age, 25% were tested between ages 4 and 8, and the remaining 43% were tested outside these time frames (Figure 3).

### 3.3 Audimetric testing

NBHS results were available for 140 patients: 55 (39.2%) patients referred bilaterally, 17 (12.1%) referred unilaterally, and 68 (48.5%) passed. Audimetric results were available for 174 patients. However, due to incomplete or inconclusive testing, hearing loss type was not defined for one patient, and laterality was not defined for three patients. With respect to hearing loss severity, 32 of 184 (17.4%) patients had mild loss, 59 (32.1%) had moderate loss, 12 (6.5%) had moderately severe loss, 25 (13.6%) had severe loss, 45 (24.5%) had profound loss, and 11 (6.0%) had hearing within normal limits (Figure 4). With respect to hearing loss type, 157 of 183 (85.2%) had SNHL alone, 1 (0.5%) had conductive hearing loss alone, 15 (8.2%) had mixed loss, and 11 (6.0%) had normal results (Figure 5). With respect to laterality of hearing loss, 102 of 180 (56.4%) had symmetric loss, and 39 (21.5%) had asymmetric loss, 31 (17.1%) had unilateral loss (Figure 6).

### 3.4 Genetic testing results

With respect to genetic test results (Table 1), 24 patients (11.8%) had genetic test results that explained confirmed SNHL (ie, “positive” tests): 19 patients (9.3%) had biallelic, pathogenic variants in GJB2 known to cause nonsyndromic SNHL (DFNB1); 1 patient (0.5%) had a heterozygous variant in GJB2 associated with syndromic SNHL (Palmoplantar Keratoderma and Deafness); and 4 patients (2.0%) had biallelic variants in GJB2 suspected to cause nonsyndromic SNHL (Likely DFNB1). One patient in the “Likely DFNB1” group also had a heterozygous mutation in a mitochondrial gene (MT-RNR1) associated with SNHL from aminoglycoside exposure. One patient (0.5%) had a heterozygous GJB2 variant of uncertain significance as well as the same heterozygous MT-RNR1 mutation noted above. The remaining 179 patients (87.8%) had no identifiable mutations in the genes tested that are associated with SNHL: 13 patients (6.4%) were identified as carriers for GJB2 mutations associated with DFNB1; 17 patients (8.3%) had benign polymorphisms in GJB2; and 149 patients (73.0%) had no variants in the genes tested. There were no patients identified with mutations in GJB6 or SLC26A4. Both in-house and send-out tests were negative for the four patients who had both types of testing ordered. Eight patients with normal hearing underwent genetic testing: four patients who passed NBHS were tested within the first days of life while in the NICU. Rationale for genetic testing could not be determined from the medical record. An additional three patients had initial testing (ie, OAEs, ABR, audiometry) that showed hearing loss, but further testing demonstrated normal hearing. Finally, one patient had genetic testing done despite normal audiometry due to an extensive family history of SNHL. None of these eight patients had pathogenic mutations in the genes tested.

### 3.5 Interventions

Of the 174 patients with confirmed hearing loss, 163 (93.7%) received intervention: 128 (73.6%) were given hearing aids, 25 (14.4%) underwent cochlear implantation, 3 (1.7%) received an FM system, and
In this study, we evaluated whether an in-house testing protocol can improve the efficiency, and ultimately the utility, of genetic testing for inherited SNHL. We demonstrated a significant decrease in average turnaround time with implementation of in-house testing (18.9 vs 53.7 days). We also demonstrated a substantial decrease in the range of turnaround times, again favoring in-house testing (7-43 vs 17-142 days). In addition, in-house testing reduced cost by $942, which translated into approximately $94 200 in overall savings in health care expenditures. Without access to pricing details at outside labs, it is difficult to explain this cost difference. However, we made concerted efforts to minimize price inflation when determining the cost of the tests and their clinical interpretation. Furthermore, in-house testing was generally more extensive in terms of the number of genes analyzed (ie, most send-out testing did not evaluate for variants in SLC26A4). These differences in turnaround times, cost, and testing scope are relevant to institutions considering implementation of in-house genetic testing for SNHL. Current guidelines set forth by the American Academy of Pediatrics Joint Committee (AAP-JC) on Infant Hearing (JCII) recommend universal screening for hearing loss before 1 month of age, diagnosis before 3 months, and intervention by 6 months.21 Given this narrow time frame, timely testing results are critical to ensure appropriate management of children with SNHL, especially children with profound SNHL who will not benefit significantly from early hearing aid use. Even with the limited testing panel described here, time frames reaching the upper limit of send-out testing (142 days) could conceivably cause unnecessary delays in management, including preventative counseling for patients with mutations in SLC26A4 and MTRRN1, sibling evaluation, and preparation for cochlear implantation. As testing panels expand, and as earlier interventions become possible (eg, cochlear implantation in children under 12 months of age), early, accurate diagnosis becomes increasingly important.22 Our study demonstrates that in-house genetic testing can consistently provide results within the time frame recommended by the AAP-JC. It is conceivable that early genetic testing in conjunction with appropriate, confirmatory audiometric testing could provide sufficient evidence to proceed with cochlear implantation as early as 6 months, and hearing aid fitting even earlier than the 6 months recommended by the AAP-JC. Based on growing evidence that neural connections associated with sensory and linguistic development develop in a sequential fashion and peak before 10 months of age, earlier intervention could provide superior benefit to hearing impaired children.23,24 Additionally, the study highlights the potential for universal, large panel, genetic screening of newborns to provide even earlier diagnosis and intervention, as well as guidance for additional testing in patients with mutations associated with syndromic loss or other organ system pathology that may not be apparent in the first months of life (eg, imaging in patients with Pendred syndrome or SLC26A4 mutations, ophthalmology evaluation in patients with Usher syndrome, cardiology evaluation in patients with Jervell and Lange-Nielsen syndrome). Given our findings, it is conceivable that these could be accomplished by as early as 1 month of age. This shorter time frame is also an important consideration given the emerging prospects of treating inherited SNHL with targeted gene therapy.25 Such interventions could improve the treatment of inherited SNHL in pediatric populations, but they would require early genetic testing to identify appropriate candidates.

Our study also demonstrated that both bilateral NBHS referral and symmetric SNHL of any severity were associated with positive genetic testing for mutations known or suspected to cause SNHL. Multiple studies have demonstrated strong associations between bilateral referral and bilateral hearing loss whereas others have shown the high likelihood for inherited hearing loss to affect both ears equally.26-29 Our study reiterates and quantifies these associations specifically within the context of evaluating children with hearing loss. For clinicians, NBHS results and/or simple audiometry alone can help

1 (0.6%) received a soft-band bone conduction hearing aid. Additionally, six patients (3.4%) with either mild or moderate loss were monitored with interval audiograms, five (2.9%) patients received no intervention, and 5 (2.9%) were lost to follow-up or transferred care before intervention was implemented.

### 3.6 Audiometric correlations with genetic testing results

We further analyzed available audiometric data to determine correlations with genetic testing results. When NBHS results were analyzed, we found that 27.3% of patients with bilateral referral tested positive for mutations associated with inherited SNHL. This compares to a 5.9% positive rate with unilateral referral, a 7.4% rate with a passed NBHS and a 5.0% rate for those who had no NBHS results. The differences between these groups were statistically significant \((P < .001)\) (Figure 7). When hearing loss laterality was analyzed, we found that 19.6% of patients with symmetric SNHL of any severity tested positive for mutations associated with inherited SNHL. This compares to 4.3% of patients with either asymmetric or unilateral SNHL of any severity. These differences were statistically significant \((P = .01)\) (Figure 8). When hearing loss severity was analyzed, there was no significant difference between positive or negative test results when comparing all levels of SNHL severity \((P = .39)\). Similarly, there was no significant difference between positive or negative genetic test results for patients with profound SNHL compared to all other levels of severity combined as a single group \((P = .60)\).

Patients with negative testing had higher rates of mixed hearing loss compared to patients who had positive genetic testing (9.4% vs 4.3%). However, a large majority of these patients (89.9%) had SNHL alone, and the overall differences between types of hearing loss seen between each group was not statistically significant \((P = .74)\). In addition, it should be noted that genetic testing was negative for patients with either bilateral referral, 17-142 days). In addition, in-house testing reduced cost by $942, which translated into approximately $94 200 in overall savings in health care expenditures. Without access to pricing details at outside labs, it is difficult to explain this cost difference. However, we made concerted efforts to minimize price inflation when determining the cost of the tests and their clinical interpretation. Furthermore,
identify patients who are most likely to benefit from early genetic testing. Indeed, given the strength of its association with positive genetic testing seen in our study, bilateral referral alone may be sufficient justification to order testing. However, it should be noted that 7.4% of patients in our study who passed NBHS later had audiometry showing SNHL and positive genetic testing for mutations in GJB2 consistent with a definite or likely diagnosis of DFNB1. This underscores the limitations of current NBHS testing thresholds, which can fail to identify some mild and potentially progressive SNHL.

Despite the potential benefits of universal genetic testing in newborns for SNHL, several potential problems, and pitfalls remain. First, as with any genetic testing, there are ethical considerations regarding patient autonomy and the potential for discrimination. In the pediatric setting, there is also the potential for causing unnecessary parental anxiety. This was described anecdotally by several of the clinicians in our study, who noted that many parents declined testing despite appropriate counseling. Second, due to the heterogeneity of mutations and wide range of inherited SNHL severity, genetic testing may identify pathogenic mutations in patients who suffer only very mild SNHL. This will likely require significant changes to and likely expansion of the protocols, education, testing, monitoring, and interventions needed for treating patients. In terms of expanded testing, many institutions are now able to test for over 100 genes known to cause hearing loss, including our own, which at the time of this publication is able to test for 157 such genes. Finally, there is the risk of false assurance from a negative test. Despite its high degree of specificity and sensitivity, genetic testing is unlikely to identify and diagnose all patients at risk for inherited SNHL, especially for patients possessing rare pathogenic mutations. As our own data demonstrated, genetic testing was negative in a majority of patients ultimately diagnosed with some form of hearing loss. Given the expanding catalog of mutations associated with SNHL and the limited number of mutations tested in this study, it is possible that some of these patients have a form of inherited hearing loss that is currently undefined or that falls outside the scope of our testing protocol.

This study has several limitations, including retrospective design and short-term observations. Small sample size was also a limitation given the frequency of certain well-established mutations. Not surprisingly, the vast majority of positive tests involved mutations in GJB2. However, we identified no mutations involving other commonly affected genes, such as SLC26A4, which accounts for anywhere from 5% to 10% of hereditary hearing loss. It is possible that the rapid turnaround times seen in our lab might not be replicable elsewhere, especially outside of larger tertiary health care centers. Additionally, our testing was limited to a narrow range of genes and mutations commonly associated with hearing loss. Although the diagnostic yield and utility of such testing would be expected to increase as more mutations are included, it is unclear how such changes would affect the overall turnaround time and cost. Furthermore, although we did develop an internal algorithm for ordering genetic testing, this was not implemented until 2016. Thus, the criteria for genetic testing varied over time as well as across the different clinical services ordering the tests. Finally, the heterogeneity of audiometric data may have limited our ability to better characterize relationships between hearing loss and genetic testing results. However, the associations identified between positive genetic tests and bilateral NBHS referral as well as symmetric SNHL could help clinicians maximize the diagnostic yield of genetic testing.

5 | CONCLUSION

In conclusion, we demonstrated that in-house genetic testing for SNHL significantly reduces testing turnaround times, thus lowering the potential for delayed diagnosis and treatment and opening the possibility for an even more expedited time frame for identifying and managing inherited SNHL. In addition, we identified two simple audiometric parameters (ie, bilateral NBHS referral and audiometry showing symmetric SNHL) that appear to increase diagnostic yield of genetic testing. Because these measures are either usually available during the neonatal period or easily acquired thereafter, they could be used by clinicians to identify patients most likely to benefit from genetic testing.

As genetic testing continues to become a more routine component of neonatal and pediatric screening with large, diagnosis and management of inherited SNHL will likely become increasingly dependent on NBHS augmented by expanded or universal genetic screening. Although such changes are not without potential practical and ethical complications, there are clear benefits to patients in terms of definitive diagnosis, more detailed prognosis, timely management, parental counseling, and avoidance of environmental factors that contribute to late onset or progressive SNHL. This report may help other institutions seeking to expand genetic SNHL testing and/or develop their own in-house testing capabilities. We view our results as an important step toward the broader goal of contributing to a goal of universal genetic screening for congenital SNHL.

ACKNOWLEDGEMENTS

We acknowledge David Dimmock and LeAnne Noll for their assistance in developing the genetic hearing test protocol. The testing protocol described in this paper was funded in part by the American Hearing Research Foundation.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

ORCID

Karl W. Doerfer https://orcid.org/0000-0002-9409-4946
Christina L. Runge https://orcid.org/0000-0003-0330-5857

BIBLIOGRAPHY

1. Morton CC, Nance WE. Newborn hearing screening—a silent revolution. N Engl J Med. 2006;354(20):2151-2164. https://doi.org/10.1056/NEJMra050700.
2. Papacharalampous GX, Nikolopoulos TP, Davili DI, Xenellis IE, Korres SG. Universal newborn hearing screening, a revolutionary diagnosis of deafness: real benefits and limitations. *Eur Arch Otorhinolaryngol.* 2011;268(10):1399-1406. https://doi.org/10.1007/s00405-011-1672-1.

3. Yoshinaga-Itano C, Apuzzo ML. Identification of hearing loss after age 18 months is not early enough. *Am Ann Deaf.* 1998;143(5):380-387.

4. Holden-Pitt L, Diaz J. Thirty years of the annual survey of deaf and hard of hearing children and youth: a glance over the decades. *Am Ann Deaf.* 1998;143:72-76.

5. Yoshinaga-Itano C, Apuzzo ML. The development of deaf and hard of hearing children identified early through the high-risk registry. *Am Ann Deaf.* 1998;143(5):416-424.

6. Yoshinaga-Itano C, Sedey AL, Coulter DK, Mehl AL. Language of early- and later-identified children with hearing loss. *Pediatrics.* 1998;102(5):1161-1171.

7. Yoshinaga-Itano C. Early intervention after universal neonatal hearing screening: impact on outcomes. *Ment Retard Dev Disabil Res Rev.* 2003;9(4):252-266. https://doi.org/10.1002/mrdr.10088.

8. Nelson HD, Bougatsos C, Nygren P. Universal newborn hearing screening: systematic review to update the 2001 US preventive services task force recommendation. *Pediatrics.* 2008;122(1):e266-e276. https://doi.org/10.1542/peds.2007-1422.

9. Watkin PM, Baldwin M. Identifying deafness in early childhood: requirements after the newborn hearing screen. *Arch Dis Child.* 2011;96(1):62-66. https://doi.org/10.1136/adc.2010.185819.

10. Chan DK, Schirijer I, Chang KW. Connexin-26-associated deafness: phenotypic variability and progression of hearing loss. *Genet Med Off J Am Coll Med Genet.* 2010;12(3):174-181. https://doi.org/10.1097/GIM.0b013e3181d0d42b.

11. Chan DK, Chang KW. GJB2-associated hearing loss: systematic review of worldwide prevalence, genotype, and auditory phenotype. *Laryngoscope.* 2014;124(2):E34-E53. https://doi.org/10.1002/lary.24332.

12. Wu C-C, Tsai C-H, Hung C-C, et al. Newborn genetic screening for hearing impairment: a population-based longitudinal study. *Genet Med.* 2017;19(1):6-12. https://doi.org/10.1038/gim.2016.66.

13. Greinwald J, de Alarcon A, Cohen A, et al. Significance of unilateral enlarged vestibular aqueduct: unilateral enlarged vestibular aqueduct. *Laryngoscope* 2013;123(6):1537-1546. doi:https://doi.org/10.1002/lary.23889.

14. Mutai H, Watabe T, Kosaki K, Ogawa K, Matsunaga T. Mitochondrial mutations in maternally inherited hearing loss. *Eur J Med Genet.* 2014;12(3):174-181. https://doi.org/10.1097/MEO.0000000000001677.

15. Mafong DD, Shin EJ, Lalwani AK. Use of laboratory evaluation and radiologic imaging in the diagnostic evaluation of children with sensorineural hearing loss. *Laryngoscope.* 2002;112(1):1-7. https://doi.org/10.1097/00005537-200201000-00001.

16. Abdurehim Y, Lehmann A, Zeitouni AG. Predictive value of GJB2 mutation status for hearing outcomes of pediatric cochlear implantation. *Otolaryngol Neck Surg.* 2017;157(1):16-24. https://doi.org/10.1177/0194599817697054.

17. Popov TM, Stancheva I, Kachakova DL, et al. Auditory outcome after cochlear implantation in patients with congenital nonsyndromic hearing loss: influence of the GJB2 status. *Otol Neurotol.* 2014;35(8):1361-1365. https://doi.org/10.1097/MAO.0000000000000348.

18. Cullen RD, Buchman CA, Brown CJ, et al. Cochlear implantation for children with GJB2-related deafness. *Laryngoscope.* 2004;114(8):1415-1419. https://doi.org/10.1097/00005537-200408000-00019.

19. The Cost of Sequencing a Human Genome. National Human Genome Research Institute (NHGRI). https://www.genome.gov/27565109/

20. Bourchany A, Thauvin-Robinet C, Lehalle D, et al. Reducing diagnostic turnaround times of exome sequencing for families requiring timely diagnoses. *Eur J Med Genet.* 2017;60(11):595-604. https://doi.org/10.1016/j.ejmg.2017.08.011.

21. Joint Committee on Infant Hearing. Year 2007 position statement: principles and guidelines for early hearing detection and intervention programs. *Pediatrics.* 2007;120(4):898-921. https://doi.org/10.1542/peds.2007-2333.

22. McKinney S. Cochlear implantation in children under 12 months of age. *Curr Opin Otolaryngol Head Neck Surg.* 2017;25(5):400-404. https://doi.org/10.1097/MO.0000000000000400.

23. Nelson CA, De Haan M, Thomas KM. Neuroscience of Cognitive Development: The Role of Experience and the Developing Brain. Hoboken, NJ: Wiley; 2006.

24. Knudsen EI. Sensitive periods in the development of the brain and behavior. *J Cogn Neurosci.* 2004;16(8):1412-1425. https://doi.org/10.1161/0898929042304796.

25. Lustig LR, Akil O. Cochlear gene therapy. *Curr Opin Neurol.* 2012;25(1):57-60. https://doi.org/10.1097/WCO.0b013e32834f038c.

26. Wood SA, Sutton GJ, Davis AC. Performance and characteristics of the Newborn Hearing Screening Programme in England: the first seven years. *Int J Audiol.* 2015;54(6):353-358. https://doi.org/10.3109/14992027.2014.989548.

27. Vila PM, Lieu JEC. Asymmetric and unilateral hearing loss in children. *Cell Tissue Res.* 2015;361(1):271-278. https://doi.org/10.1007/s00441-015-2208-6.

28. ACMG Working Group on Update of Genetics Evaluation Guidelines for the Etiologic Diagnosis of Congenital Hearing Loss; for the Professional Practice and Guidelines Committee. *Am J Med Genet.* 2014;16(4):347-355. https://doi.org/10.1002/ajmg.24152.

29. National Workshop on Mild and Unilateral Hearing Loss: Workshop Proceedings; 2005.

How to cite this article: Doerfer KW, Sander T, Konduri GG, Friedland DR, Kerschner JE, Runge CL. Development of in-house genetic screening for pediatric hearing loss. *Laryngoscope Investigative Otolaryngology.* 2020;5:497–505. https://doi.org/10.1002/liao.2379.