ORIGINAL ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Outcomes of Evaluation and Testing of 660 Individuals With Hearing Loss in a Pediatric Genetics of Hearing Loss Clinic

Devanshi Mehta,[1] Sarah E. Noon,[1] Emily Schwartz,[1] Alisha Wilkens,[1] Emma C. Bedoukian,[1] Irene Scarano,[1] E. Bryan Crenshaw III,[2,3] and Ian D. Krantz[1,4]*

[1]Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

[2]Center for Childhood Communication, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

[3]Department of Otorhinolaryngology: Head and Neck Surgery, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

[4]The Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

Manuscript Received: 18 April 2016; Manuscript Accepted: 7 July 2016

Hearing loss is a relatively common condition in children, occurring in approximately 2 out of every 1,000 births with approximately 50% of reported diagnoses having a primary genetic etiology. Given the prevalence and genetic component of hearing loss, coupled with a trend toward early diagnosis with the institution of universal newborn hearing screening, The Genetics of Hearing Loss Clinic was established at The Children's Hospital of Philadelphia to manage the diagnosis, testing, and genetic counseling for individuals and families. This paper described a cohort of 660 individuals with a diagnosis of hearing loss evaluated between July 2008 and July 2015 in the Genetics of Hearing Loss Clinic. To elucidate the cause of hearing loss in this cohort for better management and prognostication, testing included single nucleotide polymorphism chromosomal microarray, hearing loss next generation sequencing panel, and additional clinical tests inclusive of thyroid and renal function studies, temporal bone magnetic resonance imaging, and electrocardiogram. Of those evaluated, most had bilateral sensorineural hearing loss, occurring in 489/660 (74%). Additionally, 612/660 (93%) of patients presented with a nonsyndromic form of hearing loss (no other observed clinical findings at the time of exam), of which pathogenic mutations in *GJB2* were most prevalent. Of the individuals with syndromic manifestations (48/660), Usher and Waardenburg syndrome were most commonly observed. A family history of hearing loss (first degree relative) was present in 12.6% of families with available information. Through molecular analyses, clinical examination, and laboratory testing, a definitive etiologic diagnosis was established in 157/660 (23.8%) of individuals.

Key words: hearing loss; gap junction beta-2 (*GJB2*); syndromic; isolated; sensorineural

How to Cite this Article:
Mehta D, Noon SE, Schwartz E, Wilkens A, Bedoukian EC, Scarano I, Crenshaw III EB, Krantz ID. 2016. Outcomes of evaluation and testing of 660 individuals with hearing loss in a pediatric genetics of hearing loss clinic.
Am J Med Genet Part A 9999A:1–8.

INTRODUCTION

Hearing loss (HL) is a common condition in children. It is estimated that sensorineural HL (SNHL), implying dysfunction or deficiency of the cochlea, or auditory nerve, occurs with an incidence of 0.1–0.2% in neonates (severe to profound), increasing to 0.6% when the full range of loss (mild to profound) is considered [Morton, 1991; Mehl and Thompson, 2002; Morton and Nance, 2006; Hilgert et al., 2009]. The causes of HL can be broadly divided into genetic and non-genetic or acquired factors. With improved prenatal and early postnatal care, the majority of occurrences of HL in prelingual children are of genetic etiology (50–60%). This group

Conflicts of interest: None declared.
Sarah E. Noon and Devanshi Mehta contributed equally to this work.
Grant sponsor: NHGRI; Grant number: UO1HG006546; Grant sponsor: NIDCD; Grant number: R24DC012207.
*Correspondence to:
Ian D. Krantz, Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104.
E-mail: ian2@mail.med.upenn.edu
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 00 Month 2016
DOI 10.1002/ajmg.a.37855

can be subdivided into children with and without syndromic diagnoses. Nonsyndromic HL accounts for 70% of children with genetic HL and these children generally have no other physical findings or systemic clinical manifestations. Conversely, children with syndromic HL, seen in 30% of individuals, have other physical findings and systemic involvement in addition to hearing impairment. Approximately 500 syndromes have been associated with HL [Grindle, 2014]. Of the nonsyndromic HL group, the majority (75–80%) is autosomal recessive in inheritance. Autosomal dominant (15–24%), X-linked (1–2%), and mitochondrial (<1%) inheritance account for the remainder of the genetic nonsyndromic HL group [Grindle, 2014; Smith et al., 2014; Atik et al., 2015]. It has been estimated that mutations in approximately 200–250 genes (1% of human genes) are responsible for hereditary HL [Finsterer and Fellinger, 2005; Rabbani et al., 2014; Atik et al., 2015]. Thus, far more than 80 genes with greater than 1,000 mutations and 140 loci are known to be associated with nonsyndromic HL [Atik et al., 2015]. *GJB2* located on chromosome 13q encodes for the protein connexin 26 (Cx26), which is involved in potassium ion transport processes necessary for hearing. Mutations of *GJB2* impair the transfer of potassium ions within the inner ear, disrupting depolarization which normally leads to the generation of endolymphatic potential—a necessary condition for hearing [Sanecka et al., 2016a]. Abnormalities of *GJB2* account for approximately 50% of patients with autosomal recessive HL, which makes it the most common cause of HL in infants. Hearing loss as a result of homozygous or compound heterozygous mutations in *GJB2* is generally severe to profound, prelingual, bilateral, and in one third of patients, progressive [Denoyelle et al., 1999; Cryns et al., 2004; Rao et al., 2011]. More than 110 mutations in *GJB2* have been reported, with c.35delG, p.(Gly12Valfs) most common in Caucasians (28–63% of mutant alleles), c.167delT, p.(Leu56Argfs) in Ashkenazi Jewish, and c.235delC, p.(Leu79Cysfs) in Asians [Gasparini et al., 2000; Rao et al., 2011]. Since biallelic mutations of *GJB2* are responsible for only half of the patients with autosomal recessive nonsyndromic HL, there remains a significant number with only one or no mutations in *GJB2*, thereby making screening for just this one gene insufficient [Yaeger et al., 2006]. *GJB6* is located on the same chromosome (13q) as *GJB2*, and lies adjacent to it at the DFNB1 locus. *GJB6* encodes for Connexin 30 which forms gap junctions with Connexin 26. Two deletions in *GJB6* have been documented: delGJB6-D13S1830 and delGJB6-D13S1854 [Lerer et al., 2001; del Castillo et al., 2005; Rao et al., 2011]. A mutation in *GJB2* on one allele, inherited with a deletion in *GJB6* will lead to HL and explains when *GJB2* is not inherited in a biallelic pattern. Mutations in more than 50 genes in addition to *GJB2* and *GJB6* are now associated with autosomal recessive nonsyndromic HL. In addition, mutations in 24 genes are associated with autosomal dominant HL and two with X-linked nonsyndromic HL. Mitochondrial mutation inheritance may also cause nonsyndromic HL (such as the m.1555A>G mutation, which predisposes to aminoglycoside exposure ototoxicity [Rao et al., 2011]. The second most common genetic cause of bilateral sensorineural HL (BLSNHL) is mutations in the *STRC* gene on 15q15.3 [Francey et al., 2012]. Partial and complete gene deletions (including a contiguous gene deletion that also includes the *CATSPER2* gene—which results in male infertility if homozygously deleted) as well as point mutations have been found to result in hearing loss when present in homozygous or compound heterozygous states. Mutational analysis of *STRC* is complicated by the presence of a homologous pseudogene in the region [Francey et al., 2012].

The Genetics of HL Clinic at The Children's Hospital of Philadelphia (CHOP) was established to manage the diagnosis, genetic screening, and counseling of families with children who have hearing loss. In 2006, we reported our retrospective analysis of the first 500 children seen in the clinic [Yaeger et al., 2006]. The current data reported here reports on an additional 660 children seen and evaluated in this clinic from July 2008 to July 2015. It is standard of care that, if warranted by clinical exam and medical history, all patients with HL receive a urinalysis, renal and thyroid function studies, electrocardiogram (EKG), temporal bone magnetic resonance imaging (MRI), and ophthalmological examination. We review the results of these clinical screenings and the patients' genetic test results in this cohort.

MATERIALS AND METHODS

Study Population

All individuals were seen in The Genetics of HL Clinic at CHOP between July 2008 and July 2015. There were 285 females in our cohort and 375 males. Each individual was evaluated by both a geneticist and a genetic counselor. Information on a total of 660 individuals with a confirmed diagnosis of HL (inclusive of bilateral sensorineural, unilateral sensorineural, conductive, or mixed) was accessed through extraction of clinical information from the Epic electronic medical records at our institution. Not all 660 individuals were diagnosed on the newborn hearing screen. The HL diagnosis age range of the subjects in our cohort was from newborn to 17.6 years old, with an average age of 4.1 years old. The age range and mean age of subjects diagnosed with HL was identified using the Audiological and Genetic Database (AudGenDB) which is a medical, and research database resource housed at CHOP. The database draws information from several sources, including electronic health records, audiological instruments, radiological imagery, clinical genetics results, and genomics research records, and makes them accessible to researchers using a powerful, intuitive, web-based query interface. Data from patients seen at the Genetics of HL Clinic at CHOP were downloaded from AudGenDB version 2.0 [http://audgendb.chop.edu]. Data fields downloaded included, Patient Alias, Diagnosis (ICD9), and Age at Diagnosis. The initial search provided 30,141 diagnoses from 585 unique patients. When the diagnoses were restricted to those containing "hearing loss," the data frame contained 559 unique patients with 13,390 diagnoses. The minimum age of diagnosis for each patient was determined using custom scripts written in R version 3.3.0, as well as the descriptive statistics of the resultant data.

Clinical Testing

Children with HL were recommended to receive renal and thyroid function tests, (inclusive of electrolytes, urea nitrogen, TSH, T4, and creatinine analysis) an EKG, a temporal bone MRI, and an ophthalmologic evaluation as part of a routine workup.

Molecular Testing

All patients with bilateral sensorineural hearing loss (BLSNHL) were recommended to undergo molecular genetic testing including a single nucleotide polymorphism (SNP) array and HL Next Generation Sequencing (NGS) Panel. Individuals with other types of HL also had the option of genetic testing. The SNP array, which is enriched for various regions inclusive of *STRC* and *GJB6* deletions, and copy number variants of other HL genes, is widely used in the HL workup and also routinely offered for individuals with nonsyndromic HL without evidence of cognitive or intellectual delay as many subjects seen are newborns for which the child's developmental history and cognitive functioning are still unknown. Numerous variations of different HL panels have emerged over the years as new genes were discovered that explained the etiology of HL. The CHOP HL panel was developed by the Molecular Genetics Laboratory at CHOP and includes *GJB2*, *GJB6*, select exons of the Pendred syndrome gene (*SLC26A4*), and the m.1555A>G mitochondrial mutation. The Harvard Partners OtoGenome™ test, now on its third version, is currently a panel of mutations in 87 genes known to cause both nonsyndromic and syndromic HL including mutations in 66 nonsyndromic genes, and 21 genes that can present as syndromic HL, such as Usher, Pendred, Branchio-Oto-Renal (BOR), Deafness and Male Infertility (DIS), Perrault, and Waardenburg syndromes. Three different versions of this test were developed between 2008 and 2015 as new genes associated with HL were discovered. Other HL panels included the OtoChip™ which sequences 19 genes in parallel, the University of Iowa OtoSCOPE® which uses custom-targeted sequence capture for DNA enrichment followed by massively parallel DNA sequencing of mutations in 66 genes known to cause HL, and the Connexin Test which screens for *GJB2* and *GJB6* mutations. Some individuals in our cohort did not undergo molecular testing due to no recommendations made for testing after initial clinical evaluation, insurance denials, or because the family made the choice to not pursue testing for personal reasons.

RESULTS

A total of 660 individuals were evaluated in this study (Fig. 1). Of these, 489 (74.1%) had BLSNHL and 150 (22.7%) individuals had unilateral sensorineural HL (ULSNHL). There were 10 (1.5%) conductive HL subjects of which five had bilateral conductive HL and five had ULSNHL. Eleven (1.7%) patients were reported to have mixed HL, in which an individual's HL included both sensorineural and conductive components.

Of the 660 individuals seen, 612 (92.7%) had a nonsyndromic form of HL and 48 (7.3%) had a syndromic etiology for their HL, which was determined through physical exam, medical history, and/or positive genetic test results. Of the syndromic individuals, 42 (88%) had BLSNHL, 2 (4%) had ULSNHL, and 4 (8%) had conductive HL, as shown in Figure 1. Thirteen different syndromic etiologies were identified (Table I). The most common diagnoses included Usher syndrome (29.2%), Waardenburg syndrome (27.1%), BOR syndrome (14.6%), and Pendred Syndrome (8.3%). Of the 48 syndromic individuals, 28 (58.3%) had positive genetic testing results that were associated with the particular diagnosis of which 27 individuals had BLSNHL and one individual had ULSNHL. All of the gene mutations found to be causative of syndromic HL in these patients have been identified previously, including a *GATA3* mutation which was recently associated with hypoparathyroidism, sensorineural deafness, and renal disease (HDR syndrome) [Lin et al., 2015]. Sixteen individuals had normal genetic testing results, but presented with systemic involvement, dysmorphic features, and MRI that were associated with a syndromic diagnosis for which a genetic etiology remains unknown. Four individuals that were classified as syndromic did not have additional genetic testing specific for hearing loss due to past relevant medical history and a known syndromic association with HL (Down syndrome, Kawasaki disease, Sickle cell disease, and Waardenburg syndrome).

Connexin Mutational Analysis

Of the 430/612 nonsyndromic individuals screened for *GJB2* and *GJB6* mutations, at least one mutation in *GJB2* was found in 96 individuals, of which pathogenic homozygous or compound heterozygous *GJB2* mutations were identified in 70 patients. Pathogenic mutations in *GJB2*/*GJB6* (including a *GJB2* mutation and *GJB6* deletion) were found in two patients. Overall, 72/430 individuals (16.7%) had a confirmed etiology for their nonsyndromic HL due to mutations in *GJB2* and *GJB6*. This number reflects the percent positive out of our total cohort. Of the individuals with biallelic mutations in *GJB2*, 69 individuals had BLSNHL and one individual had bilateral mixed HL, as shown in Figure 1. The most common variants in the *GJB2* gene found in this population included p.(Gly12Valfs), c.101T>C p.(Met34Thr), and p.(Leu56Argfs). Two individuals in our cohort with deletions in the *GJB6* gene both had mild to moderate BLSNHL. One of these individuals was heterozygous for a c.269T>C, p.(Leu90Pro) mutation in *GJB2*, and also had a 237 kb deletion in chromosome region 13q12.11 including the *GJB6* gene; which together explain the etiology of this individual's HL. The second individual was also heterozygous in both genes, with a p.(Met34Thr) change in *GJB2* and a D13S1830 deletion in *GJB6*, explaining the cause of the patient's HL. The other 24 individuals had a heterozygous mutation in a single allele of *GJB2*, which was not sufficient to cause HL-suggesting that these individuals are carriers but likely have another etiology for their HL.

Additional Genetic Studies

There were 424/612 nonsyndromic subjects who had other genes screened in addition to connexin testing as part of a larger NGS panel or as a targeted gene screen. Of these, overall variants (including variants of unknown significance and pathogenic variants) were found in 185 (43.6%) subjects. The remaining 239/424 subjects had negative testing with no variants identified. Of the 185 individuals with variants found, 35 subjects were found to have pathogenic variants in genes other than *GJB2* and *GJB6* that confirmed a nonsyndromic etiology for their HL (*TECTA*[4], *STRC*[6], *MYO7A*, *OTOGL*[2], *SLC26A5*, *STRC/CATSPER2*[7], *TMC1*[6], *OTOA*, *MYO6*, *MYO15A*[4], *LOXHD1*, *DOCK4*). *DOCK4*, a novel gene in our analysis, is known to be expressed in the inner ear and involved in regulation of adherence junctions between cells. As shown in Figure 1, 33/35 of these individuals had

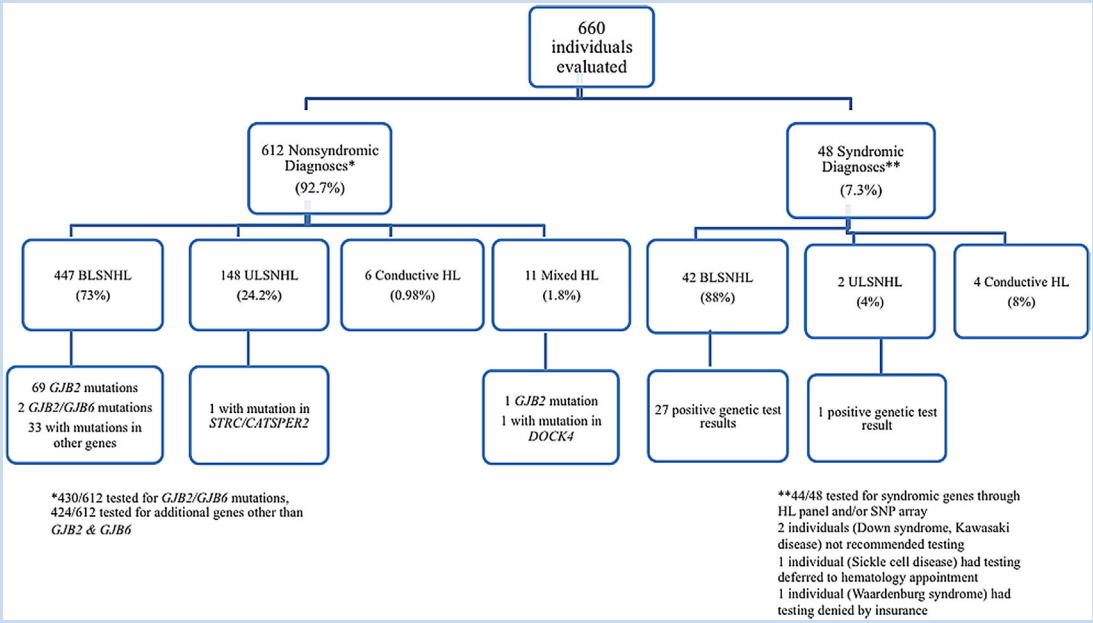

FIG. 1. Summary of hearing loss types and mutations in the cohort of 660 patients. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

BLSNHL, one had ULSNHL, and one had mixed HL. The other 150/185 patients had variants of unknown clinical significance, pathogenic variants unrelated to HL, or mutations in a recessive HL gene but present in a single allele indicating carrier status. Therefore, of all 430 nonsyndromic individuals who had testing, 107/430 (24.8%) were found to have a confirmed genetic etiology for their HL. Additionally, 617 of 660 (93.5%) individuals in our cohort received the SNP array, which can identify chromosomal abnormalities (deletions or duplications) to determine whether or not a copy number variant explains etiology for HL. A positive finding explaining HL was detected in 17/617 (2.8%) individuals, inclusive of 2 (12%) *GJB6* deletions, 4 (24%) *STRC/CATSPER2* homozygous deletions, 4 (24%) *STRC* homozygous deletions, 1 (6%) heterozygous deletion in 15q15.3 containing *STRC/CATSPER2* with a compound heterozygous point mutation in the other *STRC* allele, 1 (6%) *DOCK4* deletion, 1 (6%) *USH2A* compound heterozygous deletion, 1 (6%) 561 kb deletion in 16q11.2 linked to autism, and 3 (18%) individuals in which the SNP array identified several regions of homozygosity that overlapped with homozygous point mutations in *SLC26A4* and *USH2A*, and an 11 bp deletion inclusive of *MYO7A*.

TABLE I. Syndromic Diagnoses in Cohort of 48 Patients With Syndromic HL

Diagnosis	Number of patients (%)
Usher syndrome	14 (29.2)
Waardenburg syndrome	13 (27.1)
Branchio-oto-renal syndrome	7 (14.6)
Pendred syndrome	4 (8.3)
Deafness infertility syndrome	2 (4.2)
Goldenhar syndrome	1 (2.1)
Down syndrome	1 (2.1)
Noonan syndrome	1 (2.1)
Autism	1 (2.1)
Sickle cell disease	1 (2.1)
CHARGE syndrome	1 (21)
HDR syndrome	1 (2.1)
Kawasaki disease	1 (2.1)
Total	48

HDR, hypoparathyroidism, sensorineural deafness, and renal disease; CHARGE, coloboma, heart defect, atresia choanae, retarded growth and development, genital abnormality, and ear abnormality

Thyroid/Renal, Electrocardiogram, Temporal Bone Magnetic Resonance Imaging Testing

Thyroid and renal abnormalities, prolonged QT, enlarged vestibular aqueducts (EVA), and other inner ear abnormalities have been known to be associated with HL; therefore, evaluations for these respective findings have become part of the routine workup for HL [Cremers et al., 1998; Phelps et al., 1998; Ravecca et al., 1998; Sanecka et al., 2016b]. Among those with sufficient thyroid and renal lab results available (440), initial screening showed 121 (27.5%) had thyroid or renal abnormalities of which a high carbon dioxide level or high T4 were most common (37.2% and 23.1%, respectively), the vast majority of which were not clinically relevant and can prove to be challenging in managing these patients. For many of these individuals repeat testing was recommended to

confirm a true renal or thyroid abnormality; however, those repeat results are not available or included in this review. Among those who had EKG results available (420), 54 (12.9%) had abnormal results inclusive of sinus arrhythmia and bradycardia, heart murmur, left ventricular hypertrophy, and prolonged QT. Seven of 420 subjects who had EKG results available (1.7%) were found to have prolonged QT. All individuals with prolonged QT had BLSNHL ranging from mild-severe in severity. None of these individuals were found to have a known molecular etiology for their HL (two with variants of unknown significance, four with no mutations found, one without testing). Of those who had temporal bone imaging (CT scan and/or MRI) completed and accessible (545), 196 (36%) individuals had an abnormal finding, of which EVAs was the most common (29% of those with inner ear abnormalities). Thirty-eight of the 56 individuals with EVAs had genetic testing done, of which six patients had pathogenic variants in *SLC26A4* (16%) and two in *GJB2*. The other 30 patients had no known genetic etiology for their HL. Of the 20 individuals who had both unilateral deafness and EVA, six had genetic testing done, none of which had positive findings that explained the etiology of their ULSNHL. Thirty-one (42.4%) individuals with ULSNHL and an inner ear abnormality had severe hypoplasia or absence of a cochlear nerve. The clinical results of those with sufficient medical history by type of HL are shown in Table II. While *GJB2*/*GJB6* mutations are generally nonsyndromic, there are reports of associated systemic abnormalities [Kenna et al., 2007]. In our population with *GJB2/GJB6* related hearing loss with sufficient available clinical information, there was no significant thyroid/renal or cardiac abnormalities, although 8/57 (14%) had an abnormal temporal bone MRI imaging result inclusive of two with bilateral ostomastoid opacification, one with hypoplasia of the cochlear nerve, two with EVAs, two with underpneumatization of the mastoid cells, and one with a thickened left tympanic membrane with a large central canal defect consistent with perforation.

Family History

Six hundred individuals from 573 families were evaluated to determine the prevalence of a first degree relative affected with HL. The other 60 individuals in our cohort either did not have sufficient family history information or were adopted. Of the 573 families, 72 (12.6%) probands had a first degree family member affected with HL of which 30 probands had only a parent affected, 7 had both a parent and sibling affected, and 35 had only a sibling affected. Of the 35 probands who had only a sibling affected, 31 had molecular testing of which 14 (40%) had a known underlying genetic etiology for their HL. Six of the 14 (42.9%) had mutations in *GJB2* and the others had mutations in *SLC26A4*(2), *STRC*(1), *STRC/CATSPER2*(1), *TECTA*(2), *USH2A*(1), and *TMC1*(1). Of the individuals with BLSNHL, 19/104 (18.3%) had a positive genetic etiology and a first degree family member affected, and 63/343 (18.4%) had a first degree family member affected without a known genetic etiology for their HL.

DISCUSSION

We report our findings of 660 children seen in The Genetics of HL Clinic at CHOP from July 2008 to July 2015. This clinic was

TABLE II. Number of Patients With Clinical Abnormalities by Type of Hearing Loss

Total cohort	Thyroid/renal abnormality (%)		EKG abnormality (%)		Temporal bone MRI abnormality (%)	
Bilateral SNHL (n = 489)	110 (90.9)	T4: 23 CO2: 42 Other: 45	42 (77.8)	Prolonged QT: 7 Other: 35	110 (56.1)	EVA: 33 Cochlear nerve abnormality: 22 Other: 55
Unilateral SNHL (n = 150)	7 (5.8)	T4: 4 CO2: 1 Other: 2	9 (16.7)	Prolonged QT: 0 Other: 9	73 (37.2)	EVA: 20 Cochlear nerve abnormality: 31 Other: 22
Conductive HL (n = 10)	1 (0.8)	T4: 0 CO2: 0 Other:1	2 (3.7)	Prolonged QT: 0 Other: 2	7 (3.6)	EVA: 2 Cochlear nerve abnormality: 0 Other: 5
Mixed HL (n = 11)	3 (2.5)	T4: 1 CO2: 2 Other: 0	1 (1.9)	Prolonged QT: 0 Other: 1	6 (3.1)	EVA: 1 Cochlear nerve abnormality: 2 Other: 3
Total with sufficient clinical information	121	T4: 28 CO2: 45 Other: 48	54	Prolonged QT: 7 Other: 47	196	EVA: 56 Cochlear nerve abnormality: 55 Other: 85

HL, hearing loss; SNHL, sensorineural hearing loss; EVA, enlarged vestibular aqueducts; EKG, electrocardiogram; MRI, magnetic resonance imaging.

established in 1999 and is a genetics clinic specifically for children with hearing loss, both isolated, and syndromic in origin, where evaluation and genetic counseling are offered. We previously reported our data on 500 patients seen from 1999 to 2004 [Yaeger et al., 2006] and now provide an update, which reflects our most current experience in children with HL.

The vast majority of the 660 subjects we evaluated had BLSNHL (74.1%) followed by ULSNHL (22.7%) and <4% had conductive or mixed deficiencies. This is almost the same ratio between BLSNHL, ULSNHL, and conductive HL (3.2:1) that was in our earlier reported cohort [Yaeger et al., 2006]. McClay et al. [2008] found a ratio of 3:2 in a review of 227 children with SNHL undergoing MRI to evaluate the incidence of inner ear and intracranial abnormalities.

Overall, we found an etiology for HL in 157 of the 660 individuals evaluated (23.8%), a similar percentage (22%) as in our earlier cohort described in Yaeger et al. [2006]. These included children with a definite syndromic diagnosis (48), those with pathogenic homozygous or compound heterozygous mutations in the *GJB2* gene (70), 2 with mutations in the *GJB2/GJB6* gene, and 35 with pathogenic variants in other HL genes. This data is consistent with recent reviews [Grindle, 2014; Smith et al., 2014], which quantitate causes of prelingual HL in children. Genetic causes account for 50% of diagnoses of pediatric HL, with non-genetic (25%) and no etiology identified (25%) accounting for the remainder of patients. The individuals with HL evaluated in our clinic without a molecular etiology, may in part be due to the fact that a portion of our cohort, 175/660 (26.5%), did not receive genetic testing or results of testing were not accessible. In those who received testing, other factors that might be responsible for their HL could be copy number variants, environmental factors, or mutations in novel genes that have not yet been identified as having an association with HL.

A total of 48 individuals or 7.3% evaluated in our clinic had a syndromic etiology for their HL, with most (88%) of these having BLSNHL. These syndromic diagnoses were made through a combination of genetic testing, medical history, and physical exam by a board certified clinical geneticist/dysmorphologist. Thirteen different syndromic diagnoses were identified in our current cohort as opposed to 28 in our first series. The most common syndromic diagnoses in this cohort were Usher syndrome (29.2%), Waardenburg syndrome (27.1%), BOR syndrome (14.6%), and Pendred syndrome (8.3%) accounting for over three quarters of the syndromic cases, we evaluated as opposed to only one quarter of the syndromic cases in our original cohort in Yaeger et al. [2006]. These data correlate well with other published studies that report Waardenburg and BOR syndromes as the first and second most common types of autosomal dominant syndromic HL, with Usher and Pendred syndromes, respectively, as the first and second most common types of autosomal recessive syndromic HL, respectively [Smith et al., 2014]. Given that 30% of genetic HL is predicted to be syndromic, our numbers are lower than expected from this estimation. This once again may be due to ascertainment bias which we experienced in our first review, as some children with dysmorphic features and suspected syndromic diagnoses may have been triaged to other specialists, and evaluated in other clinics in our health system.

It is well established that with non-syndromic HL, autosomal genetic abnormalities predominate, and of this group, approximately 50% are due to mutations in the *GJB2* gene in familial occurrences. Applying the pediatric HL algorithm suggested by Smith et al. [2014] (50% Genetic × 70% Non-Syndromic × 75% Autosomal Recessive × 50% DFNB1) to our population of 430 that had molecular testing, one would arrive at 56 as an approximate number of individuals with *GJB2* mutational HL. In our population, we identified 70 individuals with homozygous or compound heterozygous mutations in *GJB2* explaining the etiology of their HL, a number within this expected range. Of children with non-syndromic HL in our study who had molecular testing, abnormalities of *GJB2* were the most frequent mutations we detected, 70/430 (16.3%). The most common genetic variants of *GJB2* were p.(Gly12Valfs), p.(Met34Thr), and p.(Leu56Argfs). Additionally, two individuals in our population had deletions in *GJB6* along with a mutation in *GJB2*. Both patients had BLSNHL and both were heterozygous for *GJB6* deletions.

Of the 185 subjects with genetic variants in genes other than *GJB2/GJB6*, 35 (18.9%) had pathogenic variants accounting for their HL. In this group we found that mutations in *STRC/CATSPER2* (9/35 25.7%), *STRC* (4/35, 11.4%), and *TMC1* (6/35, 17.1%) were most common. *STRC* and *CATSPER2* are adjacent to one another on chromosome 15 (15q15.3). *STRC* encodes for the protein stereocilin which is involved in ciliary motility in hair cells of the inner ear and when deficient may lead to problems with mechanoreception of sound waves. *CATSPER2* is involved in sperm motility. Mutations in *STRC* alone can result in non-syndromic hearing loss, while a contiguous gene deletion of both *STRC* and *CATSPER2* results in male DIS. Deficiencies in *STRC* are underdiagnosed in the HL population and our results, and others point to the importance of genetic testing at this locus [Francey et al., 2012; Hoppman et al., 2013]. *TMC1* (*transmembrance channel like 1*) encodes for transmembrane proteins and is required for normal function of cochlear hair cells. When deficient, it has been reported to cause hearing loss in various populations [Hildebrand et al., 2010; Bakhchane et al., 2015].

Additional clinical evaluations and non-genetic testing of our cohort revealed a small but not insignificant incidence of thyroid and renal abnormalities, prolonged QT on EKG, enlarged vestibular aqueduct, and hypoplasia or absence of the cochlear nerve. These results confirm such tests should, therefore, be a routine part of the HL diagnostic plan. In addition, 72 probands out of 573 (12.6%) families evaluated had a first-degree relative affected with HL, emphasizing the importance of a complete genetic family history as part of the HL diagnostic workup. Of these, there were 30 probands who had only a parent affected, 7 probands who had both a parent and sibling affected, and 35 probands who had a sibling affected with HL. Of the 35 probands who had an affected sibling, 31 probands had molecular testing, of which 14 had a known underlying genetic etiology for their HL. Six of the 14 probands (42.9%) had pathogenic *GJB2* mutations explaining the etiology of their HL. A total of 617/660 (93.5%) individuals were tested with the SNP array, of which there were 17 (2.8%) positive findings that explained HL. The most common of these pathogenic mutations included *STRC* (24%) and *STRC/CATSPER2* (24%) homozygous deletions.

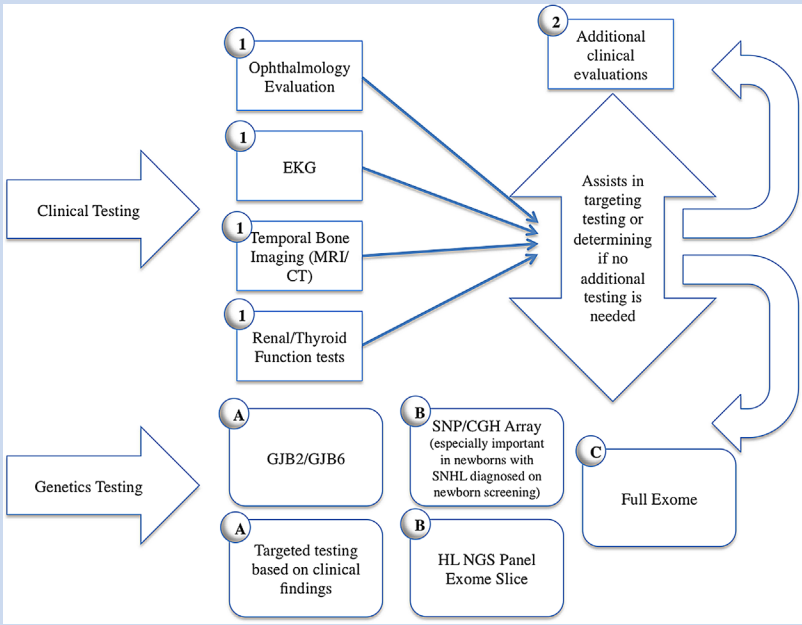

FIG. 2. Diagnostic workflow for the evaluation of individuals with sensorineural hearing loss. This workflow is primarily applicable to individuals with bilateral sensorineural hearing loss (BLSNHL). Clinical testing (i) would include baseline evaluations by Audiology and Otolaryngology with referral to: Ophthalmology (check for visual acuity and whether or not there is retinal involvement), electrocardiogram (EKG) to rule out prolonged QT, temporal bone magnetic resonance imaging (MRI), and basic chemistry and thyroid function labs to evaluate for renal or thyroid involvement. Referral to Genetics for evaluation of clinical features suggestive of syndromic forms of hearing (especially critical in neonatal and pre-toddler period where developmental and dysmorphic features may not be overtly manifesting), and to help guide genetic and genomic diagnostics. Initial genetic evaluation in the apparently nonsyndromic individual should start with *GJB2/GJB6* testing or targeted testing as suggested by the clinical evaluation (A). If within normal limits next level testing (B) should include a microarray for detection of copy number abnormalities and broader molecular genetic testing which may be inclusive of next generation sequencing (NGS) panels or exome slices. If this testing is within normal limits consideration for full exome testing (C) should be considered if a genetic contribution is suspected. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

In summary, we found in a cohort of 660 children presenting to our hearing loss clinic that BLSNHL was most prevalent (74%) and the majority of subjects presented with a form of non-syndromic hearing loss (92.7%), of which pathogenic mutations of *GJB2* were most common 70/430 (16.3%). An etiologic diagnosis (both syndromic and nonsyndromic) was obtainable in roughly one quarter of patients, through genetic analysis, clinical exam, and laboratory testing. Based on our findings, we recommend that individuals with suspected nonsyndromic BLSNHL have *GJB2/GJB6* testing or targeted testing as suggested by clinical evaluation first and if positive, no further testing is needed (Fig. 2). If negative, testing in the following order can be completed: array (especially in newborns where there is limited developmental and medical history known), next generation sequencing HL panel, exome slice for HL, and full exome sequencing. Abnormal routine labs can help direct the focus of single gene genetic testing; however, NGS panels or exome/exome slice testing may obviate the need for additional work up of EKG, temporal bone MRI, and thyroid testing. As we continue to learn more about the epidemiology and causes of HL in children, pediatric geneticists will continue to be an integral part of the evaluative team for this common sensory disorder.

ACKNOWLEDGMENTS

The authors are grateful to the individuals affected with hearing loss and family members for their kind participation. This work was supported by grant UO1HG006546 from the NHGRI (IDK, AW, IS) and AudGenDB grant R24DC012207 from the NIDCD (EBC). The authors would also like to acknowledge years of stimulating discussions, conversations, and guidance by Dr. John Carey on how to approach genetic evaluations for children with hearing loss.

REFERENCES

Atik T, Onay H, Aykut A, Bademci G, Kirazli T, Tekin M, Ozkinay F. 2015. Comprehensive analysis of deafness genes in families with autosomal recessive nonsyndromic hearing loss. PLoS ONE 10:e0142154.

Bakhchane A, Charoute H, Nahili H, Roky R, Rouba H, Charif M, Lenaers G, Barakat A. 2015. A novel mutation in the TMC1 gene causes non-syndromic hearing loss in a Moroccan family. Gene 574:28–33.

Cremers CW, Admiraal RJ, Huygen PL, Bolder C, Everett LA, Joosten FB, Green ED, van Camp G, Otten BJ. 1998. Progressive hearing loss,

hypoplasia of the cochlea, and widened vestibular aqueducts are very common features in Pendred's syndrome. Int J Pediatr Otorhinolaryngol 45:113–123.

Cryns K, Orzan E, Murgia A, Huygen PL, Moreno F, del Castillo I, Chamberlin GP, Azaiez H, Prasad S, Cucci RA, Leonardi E, Snoeckx RI, Govaerts PJ, Van de Heyning PH, Van de Heyning CM, Smith RJ, Van Camp G. 2004. A genotype–phenotype correlation for GJB2 (connexin 26) deafness. J Med Genet 41:147–154.

del Castillo FJ, Rodriguez-Ballesteros M, Alvarez A, Hutchin T, Leonardi E, de Oliveira CA, Azaiez H, Brownstein Z, Avenarius MR, Marlin S, Pandya A, Shahin H, Siemering KR, Weil D, Wuyts W, Aguirre LA, Martin Y, Moreno-Pelayo MA, Vilamar M, Avraham KB, Dahl HH, Kanaan M, Nance WE, Petit C, Smith RJ, Van Camp G, Sartorato EL, Murgia A, Moreno F, del Castillo I. 2005. A novel deletion involving the connexin-30 gene, del(GJB6-d13s1854), found in trans with mutations in the GJB2 gene (connexin-26) in subjects with DFNB1 non-syndromic hearing impairment. J Med Genet 42:588–594.

Denoyelle F, Marlin S, Weil D, Moatti L, Chauvin P, Garabedian EN, Petit C. 1999. Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexin-26 gene defect: Implications for genetic counseling. Lancet 353:1298–1303.

Finsterer J, Fellinger J. 2005. Nuclear and mitochondrial genes mutated in nonsyndromic impaired hearing. Int J Pediatr Otorhinolaryngol 69:621–647.

Francey LJ, Conlin LK, Kadesch HE, Clark D, Berrodin D, Sun Y, Glessner J, Hakonarson H, Jalas C, Landau C, Spinner NB, Kenna M, Sagi M, Rehm HL, Krantz ID. 2012. Genome-wide SNP genotyping identifies the Stereocilin (STRC) gene as a major contributor to pediatric bilateral sensorineural hearing impairment. Am J Med Genet Part A 158A:298–308.

Gasparini P, Rabionet R, Barbujani G, Melçhionda S, Petersen M, Brøndum-Nielsen K, Metspalu A, Oitmaa E, Pisano M, Fortina P, Zelante L, Estivill X. 2000. High carrier frequency of the 35delG deafness mutation in European populations. Genetic Analysis Consortium of GJB2 35delG. Eur J Hum Genet 8:19–23.

Grindle CR. 2014. Pediatric hearing loss. Pediatr Rev 35:456–464.

Hildebrand MS, Kahrizi K, Bromhead CJ, Shearer AE, Webster JA, Khodaei H, Abtahi R, Bazazzadegan N, Babanejad M, Nikzat N, Kimberling WJ, Stephan D, Huygen PL, Bahlo M, Smith RJ, Najmabadi H. 2010. Mutations in TMC1 are a common cause of DFNB7/11 hearing loss in the Iranian population. Ann Otol Rhinol Laryngol 119:830–835.

Hilgert N, Smith RJ, Van Camp G. 2009. Forty-six genes causing nonsyndromic hearing impairment: Which ones should be analyzed in DNA diagnostics? Mutat Res 681:189–196.

Hoppman N, Aypar U, Broderson P, Brown N, Wilson J, Babovic-Vuksanovic D. 2013. Genetic testing for hearing loss in the United States should include deletion/duplication analysis for the deafness/infertility locus at 15q15.3. Mol Cytogenet 6:19.

Kenna MA, Rehm HL, Robson CD, Frangulov A, McCallum J, Yaeger D, Krantz ID. 2007. Additional clinical manifestations in children with sensorineural hearing loss and biallelic GJB2 mutations: Who should be offered GJB2 testing? Am J Med Genet Part A 143A:1560–1566.

Lerer I, Sagi M, Ben-Neria Z, Wang T, Levi H, Abeliovich D. 2001. A deletion mutation in GJB6 cooperating with a GJB2 mutation in trans in non-syndromic deafness: A novel founder mutation in Ashkenazi Jews. Hum Mutat 18:460.

Lin YH, Wu CC, Hsu TY, Chiu WY, Hsu CJ, Chen PL. 2015. Identification of a novel GATA3 mutation in a deaf Taiwanese family by massively parallel sequencing. Mutat Res 771:1–5.

McClay JE, Booth TN, Parry DA, Johnson R, Roland P. 2008. Evaluation of pediatric sensorineural hearing loss with magnetic resonance imaging. Arch Otolaryngol Head Neck Surg 134:945–952.

Morton CC, Nance WE. 2006. Newborn hearing screening—A silent revolution. N Engl J Med 354:2151–2164.

Morton NE. 1991. Genetic epidemiology of hearing impairment. Ann NY Acad Sci 630:16–31.

OtoGenome™ test for hearing loss and related syndromes (87 genes) details. Partners Healthcare: Personalized Medicine.

Phelps PD, Coffey RA, Trembath RC, Luxon LM, Grossman AB, Britton KE, Kendall-Taylor P, Graham JM, Cadge BC, Stephens SG, Pembrey ME, Reardon W. 1998. Radiological malformations of the ear in Pendred syndrome. Clin Radiol 53:268–273.

Rabbani R, Tekin M, Mahdieh N. 2014. The promise of whole-exome sequencing in medical genetics. J Hum Genet 59:5–15.

Rao A, Schimmenti LA, Vestal E, Schnooveld C, Ferrello M, Ward J, Friedman B. 2011. Genetic testing in childhood hearing loss: Review and case studies. Audiol Online.

Ravecca F, Berrettini S, Bruschini L, Segnini G, Sellari-Franceschini S. 1998. Progressive sensorineural hearing loss: Metabolic, hormonal and vascular aetiology. Acta Otorhinolaryngol Ital 18:42–50.

Sanecka A, Biernacka EK, Szperl M, Sosna M, Mueller-Malesińska M, Kozicka U, Baranowski R, Kosiec A, Łazarczyk H, Skarżyński H, Hoffman P, Biefanowska K, Piotrowicz R. 2016. QTc prolongation in patients with hearing loss: Electrocardiographic and genetic study. Cardiol J 23:34–41.

Sanecka A, Biernacka EK, Sosna M, Mueller-Malesińska M, Ploski R, Baranowski R, Skarżyński H, Piotrowicz R. 2016. Evaluation of electrocardiographic parameters in patients with hearing loss genotyped for the connexin 26 gene (GJB2) mutations. Braz J Otorhinolaryngol S1808–8694(16)30044–1 [epub ahead of print].

Smith RJH, Shearer AE, Hildebrand MS, Van Camp G. 2014. Deafness and hereditary hearing loss overview. Gene Rev.

Yaeger D, McCallum J, Lewis K, Soslow L, Shah U, Potsic W, Stolle C, Krantz ID. 2006. Outcomes of clinical examination and genetic testing of 500 individuals with hearing loss evaluated through a genetics of hearing loss clinic. Am J Med Genet 140:827–836.