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RESEARCH REVIEW

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Inner ear manifestations in CHARGE: Abnormalities, treatments, animal models, and progress toward treatments in auditory and vestibular structures

Daniel I. Choo[1] | Kareem O. Tawfik[1] | Donna M. Martin[2,3] | Yehoash Raphael[4]

[1] Department of Otolaryngology—Head and Neck Surgery, University of Cincinnati College of Medicine, Cincinnati, Ohio

[2] Departments of Pediatrics, The University of Michigan Medical School, Ann Arbor, Michigan

[3] Department of Human Genetics, The University of Michigan Medical School, Ann Arbor, Michigan

[4] Department of Otolaryngology—Head and Neck Surgery, The University of Michigan Medical School, Ann Arbor, Michigan

Correspondence
Yehoash Raphael, Department of Otolaryngology—Head and Neck Surgery, The University of Michigan Medical School, MSRB-3 room 9220B, 1150 W. Med. Cntr. Dr., Ann Arbor, MI 48109–5648.
Email: yoash@umich.edu

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The inner ear contains the sensory organs for hearing and balance. Both hearing and balance are commonly affected in individuals with CHARGE syndrome (CS), an autosomal dominant condition caused by heterozygous pathogenic variants in the *CHD7* gene. Semicircular canal dysplasia or aplasia is the single most prevalent feature in individuals with CHARGE leading to deficient gross motor skills and ambulation. Identification of *CHD7* as the major gene affected in CHARGE has enabled acceleration of research in this field. Great progress has been made in understanding the role of CHD7 in the development and function of the inner ear, as well as in related organs such as the middle ear and auditory and vestibular neural pathways. The goals of current research on *CHD7* and CS are to (a) improve our understanding of the pathology caused by *CHD7* pathogenic variants and (b) to provide better tools for prognosis and treatment. Current studies utilize cells and whole animals, from flies to mammals. The mouse is an excellent model for exploring mechanisms of *Chd7* function in the ear, given the evolutionary conservation of ear structure, function, *Chd7* expression, and similarity of mutant phenotypes between mice and humans. Newly recognized developmental functions for mouse *Chd7* are shedding light on how abnormalities in *CHD7* might lead to CS symptoms in humans. Here we review known human inner ear phenotypes associated with *CHD7* pathogenic variants and CS, summarize progress toward diagnosis and treatment of inner ear-related pathologies, and explore new avenues for treatment based on basic science discoveries.

KEYWORDS
balance, CHARGE syndrome, cochlear implants, deafness, hearing, inner ear

1 | CLINICAL EVALUATION AND MANAGEMENT OF HEARING IN CS

Hearing loss is one of the most commonly recognized phenotypic features seen in CHARGE Syndrome (CS). The clinical evaluation and management of ear, hearing, and vestibular issues is critical for optimizing the potential for communicative development and function of these patients, and the degree of hearing loss is correlated with delays in receptive and expressive language development (Vesseur, Langereis, et al., 2016). In cases of CS, the overarching approach of early identification of, and early intervention for, hearing and balance conditions is often complicated by atypical anatomic features, difficulties in performing diagnostic testing in this patient population, and prioritization of life-threatening co-morbidities (e.g., cardiac or

aerodigestive conditions) that delay other tests and interventions. Underlying this complex clinical scenario is the imperative of providing optimal access to sound and speech during a critical developmental window (birth to 3-years of age) when the developing brain is most amenable to learning speech and language. Taken together, the management of auditory and vestibular issues in CS is extremely challenging and complex.

In most instances, identification of hearing loss in cases of suspected or known CS occurs early in the newborn period. The vast majority of infants with CS are now identified in the neonatal period, and birthing facilities are now associated with higher acuity newborn intensive care units where universal newborn hearing screening is included in standard protocols of postnatal care. As a result, congenital hearing losses in cases of CS are typically identified very early in the clinical course. Importantly, children born far from tertiary medical centers or those who lack access to comprehensive medical care should still be considered at risk for complications from delays in diagnosis and intervention.

The hearing loss in CS can be sensorineural (SNHL) due to anomalies of the cochlea, cochlear nerve or other inner ear structures (e.g., vestibular aqueduct) (Figures 1–5). The hearing loss also can be purely conductive (CHL) due to malformations of the external and middle ear structures (Figure 6). Very commonly, the hearing loss in CS is mixed due to a combination of inner, middle, and external ear phenotypes. Initial hearing screening methodologies in newborn intensive care settings typically employ an Auditory Brainstem Response (ABR) technique that detects hearing loss. However, when patients fail the screening newborn ABR test, best practice entails performance of a newborn hearing loss diagnostic battery that includes a more rigorous, frequency-specific ABR protocol, including otoacoustic emission testing to probe cochlear hair cell function, and immittance testing that assesses the health of the middle ear (e.g., tympanometry, etc.). Attempts are made to perform these tests in natural sleep conditions, but given the frequent anesthetics that infants with CS undergo, they may also be performed in conjunction with other procedures under anesthesia (imaging studies, cardiac testing, endoscopies, etc.) (Edwards, Kileny, & Van Riper, 2002; Edwards, Van Riper, & Kileny, 1995).

2 | COMPUTED TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING

Once the type (SNHL, CHL, mixed hearing loss) and severity of the hearing loss are established, diagnostic work-up is coordinated with a plethora of other tests and procedures that are commonly performed for individuals with CS. Best practices in CS typically include obtaining both Computed Tomography (CT) and Magnetic Resonance (MR) imaging of the inner ear and brain in order to adequately visualize the bony structural pathology and the soft tissue/neural pathologies, respectively.

CT imaging allows examination of the external auditory canal and middle ear ossicular abnormalities and is very sensitive and specific for detecting inner ear pathology. Malformations of the cochlea and vestibular system are well-demonstrated on CT, as are anomalies of the internal auditory canal. Anomalies of these structures are very common findings in the context of CS (Figures 1–5). CT imaging, however, has certain limitations that are particularly relevant in CS. For example, while the conduit for the cochlear nerve is demonstrable by CT imaging, the actual nerve is not. The cochlear nerve can be deficient or absent in CS, and the identification of cochlear nerve deficiency has great relevance in hearing intervention strategies. Finally, it should be remembered that CT involves exposure to ionizing radiation, and while most tertiary centers now employ protocols that minimize this radiation exposure (i.e., micro-dose CT), many individuals with CS require an extraordinary number of X-rays and scans that could result in a potentially unsafe cumulative exposure to radiation.

MR imaging offers extraordinary ability in demonstrating soft tissue structures and brain anomalies that are commonly seen in CS, and is an essential diagnostic tool for evaluating the inner ear for hearing (and balance) problems. Parasagittal MR images are the gold standard and offer the highest sensitivity for assessing the VIIIth cranial nerve by imaging. In contrast to CT, where only the channel for the cochlear nerve is visible, MR imaging demonstrates the nerve itself but does not permit detailed assessment of the bony conduit (Figure 4). MR is also useful in delineating cochlear hypoplasias and other malformations via demonstration of fluid in the scalae of the cochlea. MR also offers the great advantage of avoiding any exposure to ionizing radiation for these patients.

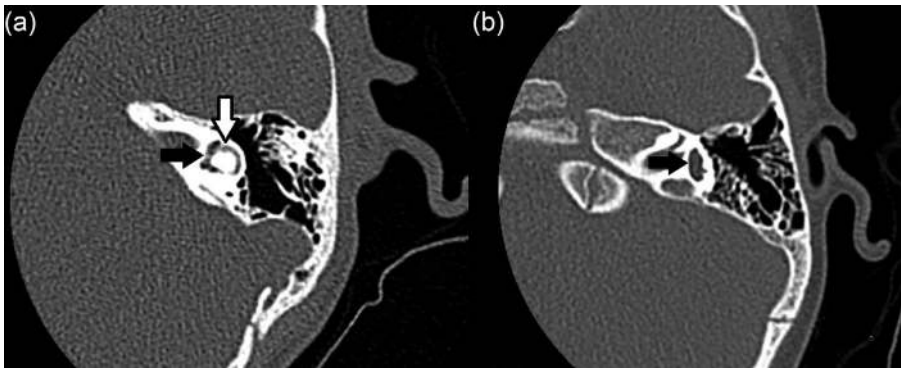

FIGURE 1 CT images demonstrating semi-circular canals in normal (a) and CS ear (b). (a) This axial CT image of a left temporal bone demonstrates a normal horizontal semicircular canal (arrow) and vestibule (asterisk). (b) In cases of CS, the horizontal semicircular canal is often absent and the vestibule (asterisk) is dysmorphic

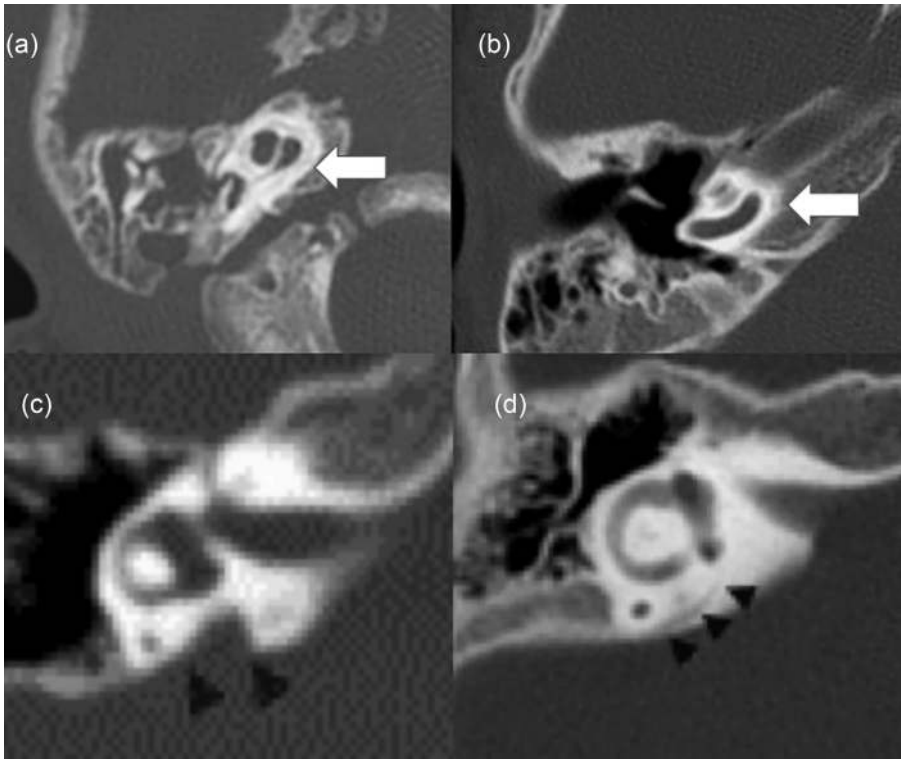

FIGURE 2 (a and b) CT images demonstrating the cochlea and the vestibular aqueduct in normal and CS ears. (a) common cochlear hypoplasia and dysplasia are indicated by the white arrow in 2A with deficient middle and apical turns. (b) A normal cochlea (white arrow), where well-differentiated 2-1/2 turns are observed. The cochlear modiolus (appearing as a hyperdense structure within the cochlea), cochlear aperture, and internal auditory canal are also demonstrated. (c and d) An enlarged (c) and normal (d) vestibular aqueduct. Black arrowheads indicate the vestibular aqueduct in each CT image

There are some important limitations to imaging for individuals with CS. Occasionally, individuals with CS will have imaging studies that suggest the absence of a cochlear nerve or a cochlea that lacks detectable innervation by cochlear nerve fibers. Yet, some of these patients may demonstrate measurable hearing by audiometric studies, suggesting the presence of neural continuity of the cochlea with functional cochlear nerve fibers. Due to the current limitations of imaging of the cochlear nerve, the term "cochlear nerve deficiency" is preferred over "aplasia" of the nerve. In such cases, clinical decision-making with respect to consideration of hearing aid and cochlear implant (CI) interventions is challenging, and no standard of care currently exists. Aural rehabilitation in these cases is discussed in more detail below.

3 | HEARING AIDS AND AUDITORY IMPLANTS IN CS

Characterization of hearing loss informs aural rehabilitative strategies, which should be instituted as soon as possible in order to optimize audiologic outcomes. In practice, however, aural rehabilitation may be delayed due to priorities of management of life-threatening medical conditions and the frequent need for prolonged hospitalizations early in life (Blake et al., 1998). Options for hearing amplification include conventional hearing aids, bone-anchored hearing aids (BAHAs), cochlear implantation (CI), and auditory brainstem implantation (ABI). Selection of an appropriate rehabilitative modality is best achieved with a

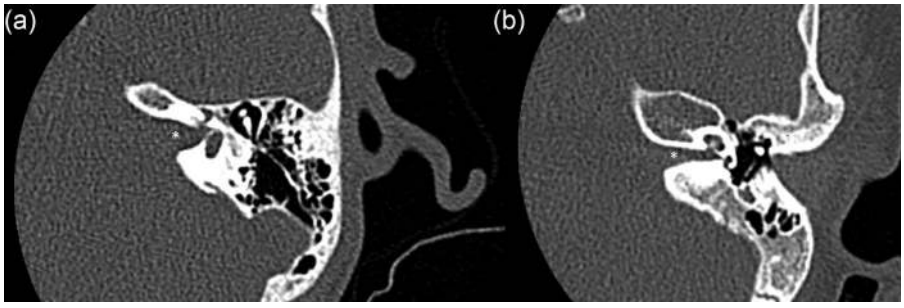

FIGURE 3 CT scans of the internal auditory canal area in a normal (a) and CS ear (b). (a) CT image of a normal left temporal bone demonstrating an internal auditory canal (asterisk) of normal caliber. (b) By contrast, this temporal bone of a CS individual demonstrates a small internal auditory canal, which may be associated with deficiencies of cranial nerves VII and VIII

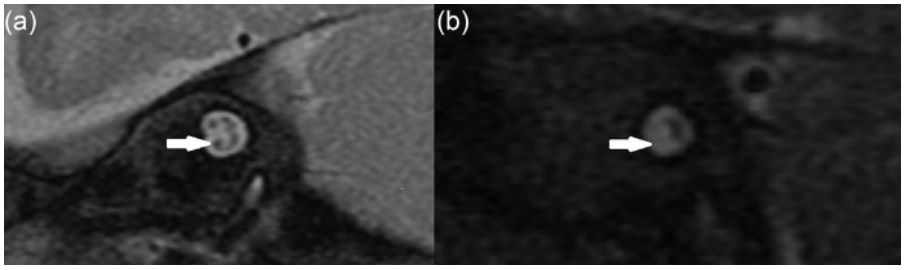

FIGURE 4 (a) A parasagittal oblique T2-weight fast-spin echo MR image demonstrates an internal auditory canal of normal caliber. The arrow points to an intact cochlear nerve, which runs anteroinferiorly within the canal. (b) In this individual with CS, the cochlear nerve is not readily distinguishable in its expected location (arrow). This is an example of cochlear nerve deficiency, a collective term used to describe hypoplasia and aplasia of the nerve

multidisciplinary team including consultants who have formal training and expertise in Audiology, Speech-Language Pathology, and Otolaryngology.

In general, conventional hearing aids may be appropriate for children with patent external auditory canals and serviceable hearing. In children with CS, however, the presence of external ear anomalies (e.g., microtia) may complicate hearing aid mold fitting. Similarly, recurrent or chronic otitis media and otorrhea are common and may limit safe use of an occlusive hearing aid mold. Episodic or progressive middle ear disease may result in changes in the severity of CHL and degree of benefit from conventional hearing aids.

In individuals with CS, the presence of CHL may often be attributable to abnormalities of the middle ear. Middle ear abnormalities in CS occur in a spectrum of severities and may affect multiple components of the bony architecture of the middle ear cleft. Occurring in up to 80% of CS ears (Ha, Ong, Wood, & Vijayasekaran, 2016), ossicular anomalies range from dysplasia to absence of the malleus, incus or stapes. Ossicular fixation may also occur, with fixation of the head of the malleus to the anterior tympanic wall being the most frequent event (Dhooge et al., 1998). Dysplasia or aplasia of the round window or oval window are also possible. Patients with conductive hearing loss, regardless of the anatomic correlate accounting for the hearing deficit, may be candidates for BAHA devices, which transmit sound to the cochlea via bone conduction. Many cases of CHL may also be treated by alternative surgical means. Ossicular chain abnormalities such as fixation or dysplasia/absence are also amenable to targeted surgical approaches, which vary depending on the specific ossicular abnormality. The goal of such an operation is to establish a mobile, continuous conductive mechanism that effectively transmits a sound wave to a functional cochlea. In certain anatomic circumstances, such as absence or dysplasia of one or more ossicles, placement of an ossicular reconstruction prosthesis may be indicated to restore coupling of the tympanic membrane to the oval window of the cochlea, resulting in significant hearing improvement. Surgical correction of conductive hearing loss is not indicated in the setting of nonserviceable hearing or medical unfitness for general anesthesia.

For children with severe to profound SNHL, CI may provide dramatically improved access to sound and speech that could not be obtained by other, more conservative, interventions. CI surgery traditionally entails performance of a mastoidectomy and facial recess approach, followed by insertion of an electrode array into the cochlear lumen. The receiver-stimulator component of the implant is situated atop the temporoparietal skull, deep to the soft tissues. After implantation, the patient applies an external speech processor to the skin overlying the receiver-stimulator. The speech processor transforms and transmits the sound information to the receiver-stimulator. In turn, the receiver-stimulator transmits the electronic signal to the electrode array, which stimulates the spiral ganglion neural elements within the cochlear modiolus.

Candidacy for CI is best determined in multidisciplinary evaluation by Audiology and Otolaryngology consultants and is typically reserved for children who have gained little or no hearing benefit with conventional hearing amplification. Although the potential for significant hearing

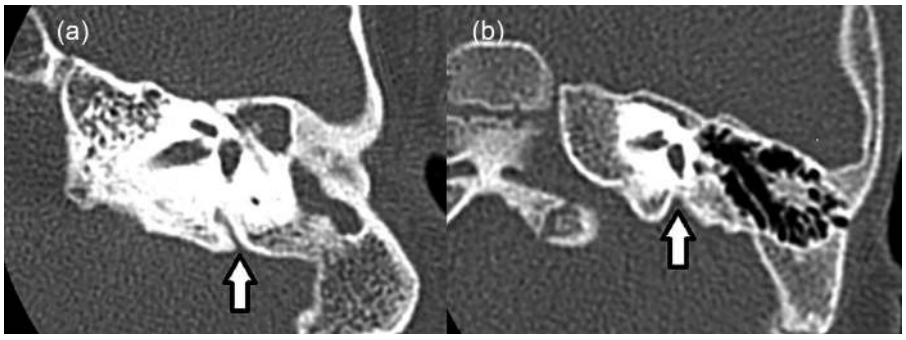

FIGURE 5 CT scans of the vestibular aqueduct in normal (a) and CS ear (b). (a) In this axial CT of a left temporal bone, the vestibular aqueduct (arrow) is of normal caliber throughout its demonstrated course. (b) In this individual with CS, the vestibular aqueduct is enlarged (arrow) throughout its demonstrated course

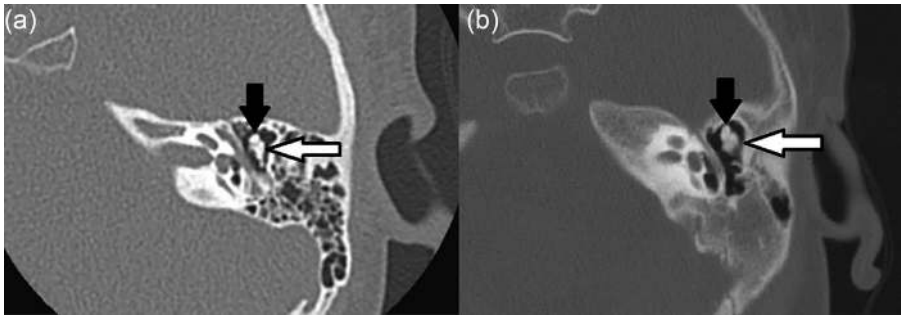

FIGURE 6 CT images demonstrating middle ear ossicular structures in normal ear (a) and in CS ear (b). (a) This axial CT image of a normal left temporal bone demonstrates a normal malleus-incus complex at the level of the epitympanum. The solid black arrow points to the head of the malleus and the white arrow to the short process of the incus. (b) In CS, dysplasia of the malleus-incus complex can be subtle. This CT image demonstrates slight abnormalities in the shape and orientation of the malleus and incus

benefit exists, outcomes after CI in CS are variable. To date, predictors of hearing outcome after CI in CS are poorly described and require further investigation. Children with CS and comorbid developmental delays, cochlear nerve deficiency, or other inner ear malformations may perform more poorly after CI (Buchman et al., 2011). Among children with cochlear nerve deficiency and hypoplastic inner ear anomalies, achievement of open-set speech recognition (i.e., understanding speech without the aid of visual clues) is rare. In a review of children with CS and *CHD7* pathogenic variants who underwent CI, a larger diameter of the cochleovestibular nerve on imaging and absence of severe cognitive developmental delay correlated with better outcomes after CI (Song et al., 2011). In turn, the authors recommend that CI be considered in individuals with CHARGE when the diameter of the cochleovestibular nerve is larger than or equal to the diameter of the facial nerve on MRI and cognitive developmental delay is not severe. Vesseur, Langereis, et al. (2016) reviewed ten children with CS who underwent CI and found that all children displayed auditory benefit and improved disease-specific quality-of-life. On comparing these children to a cohort of patients with CS and sufficient hearing without CI, the authors found that children implanted at a young age (37 months or younger) with a long period of CI use (>5 years) and minor comorbidities developed spoken language at a level comparable to children with CS and no CI. In children who are deemed to be candidates for CI, implantation should be performed early to maximize the potential for auditory benefit.

It should be noted that although multiple CI device models are now MRI-compatible, many are not FDA-approved for MRI compatibility. When considering a child's candidacy for CI, the surgeon, CI team, and family must account for the potential need for repeated imaging tests. As occurs with any metal-containing device, the receiver-stimulator portion of the CI produces a shadow on CT imaging. The magnet component of the CI also produces a shadow on MR imaging. These shadow artifacts may be problematic for patients who require serial neuroimaging to monitor progression of lesions/masses. In these cases, implantation of the ear contralateral to the lesion and/or MRI-compatible CI may be advisable, depending on each patient's anticipated imaging protocol.

CI surgery may be technically challenging in children with CS due to the abnormal temporal bone anatomy. Structures that normally serve as important anatomic landmarks during temporal bone surgery may be dysplastic or completely absent in the context of CS. In conventional CI surgery, mastoidectomy facilitates visualization of, and access to the middle ear, and basal turn of the cochlea. Once the middle ear is accessed, the tympanic segment of the facial nerve, oval window, stapes, cochlear promontory, and round window typically orient the surgeon to the expected location of the scala tympani within the basal turn of the cochlea. Vesseur, Free, et al. (2016) performed a review of temporal bone CT findings in patients with CS and cataloged the potential impact of these findings with respect to CI surgery. As the authors describe, the presence of an underdeveloped mastoid in CS may impede access to the middle ear. Similarly, the horizontal semicircular canal, an important landmark located at the medial wall of the antrum, normally indicates the approximate depth of the mastoid segment, and second genu of the facial nerve within the temporal bone. This structure, however, may be dysplastic or absent in children with CS. In this situation, the surgeon may skeletonize the tegmen mastoideum and follow it medially as it forms the superior boundary of the antrum. According to Vesseur, Free, et al. (2016) in the middle ear, the tympanic segment may take an aberrant course over the cochlear promontory (19% of ears) and also the round window (9.5% of ears), potentially impeding insertion of the CI electrode into the cochlea. The oval window and/or round window may also be atretic or otherwise underdeveloped. These anomalies pose a significant challenge when planning placement of the cochleostomy. The facial nerve, if taking an aberrant course over the promontory and round window, may be at risk during formation of the cochleostomy (Ahn & Lee, 2013) and may necessitate abortion of the procedure (Bauer, Goldin, & Lusk, 2002). Furthermore, the presence of anomalies of the oval window, round window, and/or ossicular chain may distort the surgeon's sense of the location of the scala tympani at the basal turn of the cochlea (Vesseur, Free, et al., 2016). In order to avoid surgical misadventure, it is imperative that the surgeon carefully review preoperative imaging before proceeding with surgery.

In patients with cochlear nerve deficiency who demonstrate no auditory perception or who have received but failed to benefit from CI, ABI may be an option. ABI surgery entails performance of a retrosigmoid craniotomy approach and placement of an implant electrode array next to the cochlear nucleus complex of the lateral brainstem. Similar to CI, a receiver-stimulator component is affixed atop the temporoparietal skull, deep to the soft tissues of the scalp. Historically, ABI has been reserved

for patients with Neurofibromatosis type II (NF2) and bilateral VIIIth nerve tumors. However, in recent years, off-label indications for ABI have expanded to include children with cochlear or retrocochlear pathology not amenable to rehabilitation with CI. Individuals with NF2 who have undergone ABI have typically demonstrated benefits of novel access to environmental sounds, an improved ability to monitor and adjust the volume of their voices, and enhanced lip reading (Colletti, Shannon, Carner, Veronese, & Colletti, 2009; Grayeli, Kalamarides, Bouccara, Ambert-Dahan, & Sterkers, 2008; Lundin, Stillesjo, Nyberg, & Rask-Andersen, 2016). Song et al. (2011) reported two patients with CS who underwent ABI after CI failed to confer benefit. Both patients demonstrated improved auditory perception following ABI, suggesting this procedure is a reasonable alternative to CI in the context of CS.

4 | VESTIBULAR CONSIDERATIONS IN CS

Abnormal vestibular function remains one of the more poorly studied and understood aspects of CS. In otherwise healthy individuals with isolated anomalies of the semicircular canals or vestibular aqueduct, those deficits often go unnoticed or remain "subclinical" in presentation as individuals are able to compensate with either contralateral or central mechanisms. However, in CS, those compensatory mechanisms are frequently also affected. Enlarged vestibular aqueduct may be associated with significant SNHL, which can occur suddenly or progressively. In addition, bilateral inner ear malformations, ocular colobomas that cause visual impairments, and cerebellar and brainstem malformations that impair central vestibular systems can all compound dysfunction related to the peripheral deficits and lead to delays in postural control, motor skill development, walking, and other higher order balance functions.

Children with CS often demonstrate a spectrum of inner ear vestibular anomalies on imaging (and vestibular testing) and a similarly variable picture of vestibular impairments on examination and in daily life. In a prospective cohort study of 17 children with CS, Abadie et al. (2000) found that specific anatomical vestibular anomalies consistently correlate with performance in clinical vestibular testing. Semicircular canal anomalies were associated with retardation of postural development and lack of response on evaluation of canal function. In their series, no patient with bilateral semicircular canal anomalies was able to walk before age 18 months. The authors argued that although not commonly performed in practice, vestibular functional testing may guide physiotherapy in children with CS. For example, in children with intact otolith function, vertical, and horizontal translational movement may be incorporated into therapy to aid in organization of balance. As described in multiple reports (Abadie et al., 2000; Murofushi et al., 1997), children with congenitally absent vestibular function can achieve motor milestones, presumably by compensatory use of visual and/or proprioceptive input. In line with these reports, vestibular rehabilitation therapy remains a primary treatment option for many patients with CS and offers a remarkably effective and safe intervention. Although the impact of vestibular dysfunction on global intellectual development in CS is poorly studied, one report by Raqbi et al. (2003) suggests that the presence semicircular agenesis or vestibular and otolithic deficiency does not portend poor cognitive function. As our understanding of inner ear vestibular anomalies improves (as well as the functional implications of those anomalies), so will the potential to enhance and develop novel interventions for those vestibular dysfunctions.

5 | *CHD7* GENE STRUCTURE, EXPRESSION, EVOLUTIONARY CONSERVATION, AND FUNCTION

CHD7 is the gene most commonly affected in CS. *CHD7* encodes an ATP-dependent chromatin remodeling protein and member of the chromodomain helicase DNA binding protein family (Vissers et al., 2004). In humans, *CHD7* is located on chromosome 8q12 and encompasses 188 kb of DNA. Human *CHD7* contains 38 exons, 37 of which are coding. There is a high degree of homology between *CHD7* in humans and other organisms, including mouse, zebrafish, fly, and yeast. In mouse, *Chd7* is expressed in the oocyte (Cheng et al., 2013), in embryonic stem cells (Schnetz et al., 2009), and is broadly expressed in the e7.5 embryo (Randall et al., 2009), after which time it is gradually down-regulated during embryogenesis (Bosman et al., 2005; Hurd et al., 2007). Eventually, *Chd7* expression becomes enriched in specific cells and tissues, especially in organs where malformations are known to occur in CHARGE. CHD7 is localized primarily to the nucleus, although a recent study suggested it may also localize to inner ear stereocilia (Bird et al., 2017). *Chd7* expression in mouse neural tissues likely reflects its important functions in a wide variety of peripheral and central nervous system regions. In the mouse ear, *Chd7* is highly expressed in the e9.5 otocyst and surrounding mesenchyme, and over the next 8 days of gestation becomes concentrated in VIIIth nerve ganglion neurons and in auditory and vestibular sensory epithelia (Hurd, Poucher, Cheng, Raphael, & Martin, 2010).

Like other ATP-dependent chromatin remodeling proteins, CHD7 uses the energy of ATP to slide nucleosomes along DNA or evict them (Bouazoune & Kingston, 2012). CHD7 binds to linker DNA adjacent to nucleosomes (Manning & Yusufzai, 2017). CHD7 is enriched at sites of methylated histone H3 residues at promoters and enhancers (Elkareh et al., 2009). CHD7 has been identified at over 10,000 regions of the genome in both mouse and human (Schnetz et al., 2010). CHD7 participates in large multi-protein complexes (Bajpai et al., 2010) and may act to tether other proteins such as pioneering transcription factors at key regions in the genome. Like other chromatin remodelers, CHD7 and its associated complexes likely promote open (euchromatic) chromatin and closed (heterochromatic) chromatin to activate or repress its target genes, respectively. Thus, CHD7, via its function as a chromatin remodeling factor, may have direct or indirect effects on downstream target genes.

6 | *CHD7* MUTANT MOUSE MODELS AND PHENOTYPES

The first report of *Chd7* mutations in the mouse came from the description of a series of nine different *Chd7* alleles (*Edy*, *Todo*, *Whi*, *Lda*, *Obt*, *Cycn*, *Mt*, *Dz*, *Flo*) and another allele (*Whl*) that mapped to the

same region of mouse chromosome 4 (Bosman et al., 2005). Most of these alleles had been generated using Ethyl-Nitrosourea (ENU) in a screen for mutations that result in hyperactivity and circling behaviors (Alavizadeh et al., 2001; Hawker, Fuchs, Angelis, & Steel, 2005; Kiernan et al., 2002; Nolan et al., 1995; Pau, Hawker, Fuchs, De Angelis, & Steel, 2004; Pickard, Sollars, Rinchik, Nolan, & Bucan, 1995). Each of these mutant mice harbors a heterozygous loss of function variant in the *Chd7* gene, and exhibits phenotypes including hyperactivity, head-bobbing, circling, and structural abnormalities of the semicircular canals. In addition to the inner ear phenotypes, choanal atresia, clefting, cardiac malformations, genital hypoplasia, reduced growth, and embryonic lethality at mid-gestation were also reported, strengthening the argument that these mice are an excellent model for human CS (Bosman et al., 2005).

In subsequent studies, the Martin laboratory generated a *Chd7* gene trapped allele ($Chd7^{Gt}$) that proved useful for tracking *Chd7* expression in mouse cells and tissues (Hurd et al., 2007). Analysis of $Chd7^{Gt/+}$ mice uncovered additional new phenotypes including mixed conductive and sensorineural hearing loss, middle ear structural abnormalities, and defects in highly specific regions of the vestibular system including lack of innervation to the posterior crista ampullaris (Adams et al., 2007; Hurd et al., 2007, 2011).

To overcome the embryonic lethality caused by complete loss of *Chd7*, three groups have generated and studied *Chd7* conditional deletion mice (Feng et al., 2013; Hurd et al., 2010; Jones et al., 2015). Another group created an allele that allows for recovery of *Chd7* deletion (Randall et al., 2009). Use of these mice has allowed for the discovery of new roles for *Chd7* in inner ear development, including abnormalities of the sensory epithelia in the vestibular system. A series of studies led to the novel observation that CHD7 plays a critical role in neurogenesis of the inner ear as early as e9.5 (Hurd et al., 2010). This requirement of CHD7 for proper neuronal development was also been observed in the nasal epithelium and in gonadotropin releasing hormone-expressing neurons, and may help explain the abnormal olfaction and lack of pubertal development observed in individuals with CHARGE (Layman et al., 2009; Layman, Hurd, & Martin, 2011).

7 | PLEIOTROPIC ROLES FOR *CHD7* IN NEUROGENESIS OF THE INNER EAR AND OTHER AREAS OF THE NERVOUS SYSTEM

In addition to the inner ear, CHD7 has been implicated in neurogenesis of the forebrain subventricular zone, where newly born neurons migrate rostrally to populate the olfactory bulb (Micucci et al., 2014). Loss or reduction of this progenitor population might explain why arhinencephaly (reduced size or absence of the olfactory bulbs) is a common MRI finding in CHARGE (Legendre et al., 2012). Interestingly, *Chd7* was recently shown to regulate granule cells in the cerebellum and stem cells in the hippocampus, raising the possibility that auditory and vestibular neural pathways in the brain may also be sensitive to *Chd7* loss (Feng et al., 2013, 2017; Jones et al., 2015; Whittaker et al., 2017). Use of heterozygous mutant *Chd7* mice has also led to the discovery that haploinsufficiency for *Chd7* may protect from noise-induced hearing loss, likely related to conductive hearing loss and middle ear structural abnormalities (Hurd et al., 2011).

Identification of the specific cells that express *Chd7* and/or the cells that are affected by *Chd7* loss is crucial for developing therapies. Studies of the inner ears of wild type mice have localized *Chd7* to the sensory organs and the neural elements of both cochlear and vestibular portions of the inner ear during development (Hurd et al., 2010, 2011). In sensory organs, *Chd7* is expressed in both epithelial and mesenchymal derivatives (Hurd et al., 2010). In mature ears, *Chd7* expression appears to be concentrated in sensory epithelia and in neurons, suggesting that therapies targeting these cells for therapeutic approaches may be the most efficacious.

Despite the rapid progress and new knowledge acquired about *Chd7* expression, structure, and function over the past 12 years, many outstanding questions remain. Most of our current understanding of *Chd7* function relates to roles during development. In contrast, it is not yet known whether *Chd7* has important roles in the function or integrity of mature tissue. It will be helpful to use *Chd7* conditional mutant mice to delete *Chd7* at later stages of development and in adults to determine its function(s) in mature animals. Also unknown is how CHD7 governs cochlear development, and which specific genes are involved. CHD7 may be required for proper hair cell formation, although this has not been demonstrated in heterozygous mutant mice. The CHD7-dependent intracellular and extracellular signaling pathways (i.e., *Shh*, *Bmp*, *Wnt*) involved in cochlear development and function have also not yet been identified. These are all areas poised for future study.

8 | GENERAL PROGRESS IN TREATING HEREDITARY DEAFNESS

As noted above, treatment for hereditary deafness currently relies mostly on hearing aids and CIs that address the symptoms but do not cure the disease. To design means for biological treatments, progress in basic science is needed. In general, basic science experiments relevant to CS are aimed at (1) understanding the molecular mechanisms of action of CHD7; (2) determining which cells are affected by reduced levels of CHD7; and (3) determining how to compensate for loss of CHD7. In order to design biological therapies, it is necessary to assess the efficacy of potentially therapeutic molecules and to find or develop means for delivering these molecules to the correct target cells.

Progress in several fields has enabled testing of new concepts leading toward biological cures that have relevance to CS. Areas of concentration include, (1) identification of genetic causes of hearing loss; (2) improvements in gene delivery technology; (3) advances in understanding of the biology of ear-specific genes; and (4) progress in knowledge about the outcomes of genetic disorders at the cellular and molecular levels. Progress in all these aspects of inner ear therapy could be generalizable and relevant to multiple genetic disorders; however, differences in function between genes or gene products likely will necessitate gene-specific and variant-specific efforts to convert principles into effective cures for hereditary deafness, including CS.

The complexity of accomplishing biological therapies for CS becomes evident when considering recent progress in other mouse models of human hereditary deafness. Here we review approaches using antisense nucleotides, siRNA, and a variety of viral vectors. The Smith laboratory in Iowa accomplished a pioneering success in treatment of a mouse model with dominant-negative mutations in *Gjb2* (Maeda, Fukushima, Nishizaki, & Smith, 2005). They injected *Gjb2* siRNA into the cochlea and induced downregulation of *Gjb2* expression, which partially improved hearing in these mice. Their study showed the utility of using siRNA for treating genetic deafness, and demonstrated that the function of the protein was not altered by manipulation of its level in tissues. Importantly, however, the direct relevance of this work to CS may be limited because the majority of *CHD7* pathogenic variants in humans involve reduction of protein levels.

Among hereditary forms of human hearing loss, autosomal recessive pathogenic variants in *GJB2*, the gene encoding Connexin 26, are the most common. The high incidence of these variants in the population has inspired studies on phenotypic rescue in *Gjb2* mutant mice. Two recent studies used mice with conditional deletions of *Gjb2*, one expressing panotic *Foxg1-Cre* (*Foxg1-cCx26*KO) (Yu et al., 2014), and the other expressing Cre recombinase under the control of the supporting cell-specific P0 promoter (*Cx26fl/fl;P0-Cre*) (Iizuka et al., 2015). Both studies used gene transfer with adeno-associated viral (AAV) vectors and showed that when wild-type *Gjb2* is expressed during development and maturation of mutant inner ear cells, inner ear structure, and protein function are restored, yet hearing does not significantly improve. These studies show that gene replacement can, in principle, contribute to rescue of cells and their functions, but the reasons why hearing does not improve need to be determined before attempting clinical use.

In another groundbreaking study, the Lustig laboratory published results on a mouse model for DFNA25, a form of deafness caused by loss of *VGLUT3*, a vesicular glutamate transporter, in inner hair cells (Akil et al., 2012). Using an adeno-associated virus (AAV) vector, the wild-type *Vglut3* gene was inserted into inner hair cells of developing ears and resulted in partial rescue of structure and function. If similar gene replacement can be accomplished in mature ears, it would increase the feasibility of gene replacement for clinical use. It is possible that restoring *Chd7* in specific types of cells (i.e., hair cells) could help improve their function and enhance hearing and balance for individuals with CS.

Another fascinating example of phenotypic rescue involves Jervell and Lange-Nielsen (JLN) syndrome, a disorder caused by mutations in the potassium channel subunit *KCNQ1*. Individuals with JLN present with severe congenital deafness. In a recent study, the Lin laboratory injected a modified AAV vector containing wild-type *Kcnq1* into the inner ears of developing *Kcnq1* mutant mice and determined that exogenous expression of *Kcnq1* transgene in the stria vascularis was sufficient to rescue inner ear structure and improve hearing (Chang et al., 2015).

The laboratories of Geleoc and Holt (Pan et al., 2017) reported results from a knock-in mouse model of Usher syndrome type 1C. They delivered a synthetic AAV vector, Anc80L65, containing wild-type *Ush1c* into the inner ear of *Ush1c* c.216G>A mutant mice. The Anc80L65 vector transduced hair cells with high efficiency, leading to expression of wild-type *Ush1c* and improvement of hearing and balance. Notably, this study was performed on developing mice with immature inner ears; clinical relevance and feasibility will be improved if similar outcomes are observed in mature ears.

Taken together, these studies show that gene replacement strategies can lead to convincing and impressive phenotypic rescue of hereditary deafness in mutant mice. If through design and construction of a shuttle vector system to deliver *Chd7* to the correct target cells, it is determined that mice with *Chd7* loss of function also exhibit improved auditory function with over-expression of wild-type *Chd7*, an opening for biological cures could be foreseen. Nevertheless, several hurdles and challenges for gene therapy remain and are discussed in the next section.

9 | POTENTIAL APPROACHES AND SPECIFIC HURDLES FOR DEVELOPING TREATMENT TO CHARGE

There are several caveats that need to be considered when exploring and developing treatments for inner ear abnormalities in CS. Timing of therapies is an important issue, since at the time of birth, human inner ears are mature and functional whereas mouse ears are immature. The main question that needs to be answered is whether providing functional *CHD7* in humans postnatally will be sufficient to bestow function to developmentally abnormal cells. Experiments using plasmids to introduce wild-type *Chd7* into mutant cells should provide clues, and these experiments need to be completed in animal models before clinical trials in humans could be considered. The presence of a full complement of hair cells and supporting cells in *Chd7* heterozygous mutant mice (Adams et al., 2007; Bosman et al., 2005; Hurd et al., 2011) is important, as these cells are obvious targets for therapy. If developmental loss of *Chd7* cannot be corrected by gene replacement at later (mature) stages, it may be necessary to employ intra-uterine gene therapy, which would present considerable technological challenges.

The method of gene delivery also presents a challenge. Delivery of genes into living cells in vivo requires use of a viral vector as a shuttle. The most commonly used viral vectors are AAV which can only carry a limited size gene insert. The human *CHD7* coding sequence (~9 kb) far exceeds the limits of AAV and even adenovirus vectors, in which a larger insert can be accommodated. Newer technology for splitting genes and inserting each portion into a different vector may help facilitate delivery of large genes like *CHD7*; however, the efficiency of gene transfer is reduced with these methods. One alternative to delivering the coding region is to introduce downstream effectors of CHD7, especially as some CHD7 targets may be smaller genes encoding proteins that are also secreted. While delivery of diffusible proteins may be technically easier than gene transfer to accomplish, specificity could be more challenging to achieve.

In order for gene therapy to be clinically relevant, off-target effects of therapy must also be minimized. In the best case scenario, only target cells would be transduced by gene therapy vectors or express the transgenes. Use of cell specific promoters to drive

expression of the transgene is one way to accomplish targeted gene expression. In such experiments, it is typically necessary to confirm that newly introduced genes are expressed only in the desired cells or that expression in other cells has no detrimental effects. With current knowledge about where *Chd7* is expressed, it appears that hair cells and auditory neurons may be the main targets for gene delivery in *Chd7* mutant inner ears. However, the effects of wild-type *Chd7* transgene expression in cells of *Chd7* mutant mice have yet to be tested.

Another technical challenge in gene therapy is the surgical procedure required for injecting viral vectors or other reagents directly into the cochlea. The inner ear is a complex organ containing multiple cell types from various developmental sources and embryonic origins. This heterogeneous population of cells and the intricate spatial organization of the ear complicate delivery of gene therapy vectors. Access to the inner ear is also difficult given the hard bone that surrounds and protects it. However, the ear also has some features that might facilitate gene therapy treatments. Most importantly, the perilymphatic fluid spaces (scala vestibuli and scala tympani) are continuous and most of the potential target cells are adjacent to the lumen, such that widespread delivery of molecules within the ear could be feasible.

Many individuals with CS have heterozygous nonsense pathogenic variants of *Chd7* that could be amenable to therapies to induce ribosomal read-through (Brendel, Klahold, Gartner, & Huppke, 2009). Aminoglycosides and other drugs can enable ribosomes to bypass premature termination codons and allow translation to proceed, albeit with insertion of alternative amino acids. Preliminary experiments have provided proof-of-principle for this approach, but the efficiency at present remains low (~1–5%) (Vecsler et al., 2011). Future improvements of these drugs could enhance their efficiency; however, aminoglycosides are ototoxic, and special care will be needed to avoid negative outcomes in the inner ear.

Animal models show that some viral vectors transduce hair cells with high efficiency when injected into the cochlear fluids (Askew et al., 2015; Yu et al., 2014), whereas others, especially adenovirus, are very poor at transducing mature hair cells (Kawamoto, Ishimoto, Minoda, Brough, & Raphael, 2003; Venail et al., 2007). In some cases, injections into scala media can enhance transduction of supporting cells (Ishimoto, Kawamoto, Kanzaki, & Raphael, 2002) but these may not be the optimal targets for treating CS. It should also be considered that in the human inner ear, access to the scala media is not available with current surgical technology.

A final consideration for gene therapy is that any injection of reagents into the inner ear may have significant side effects that need to be considered and compared to potential benefits. Among the many potential negative outcomes of surgical manipulation of the cochlea are perilymph fistula (persistent leakage of cochlear fluid), hearing loss, vertigo and other balance problems, pain, immune reaction, and facial nerve paresis or paralysis. It is possible that for ears with profound deafness, treatments with high efficacy will be tolerated and justified in spite of the side effects, but in cases of functional hearing, they may render the treatment impractical.

10 | CONCLUSION

Complex inner ear disorders are common in CS. Clinical imaging, therapies, and surgeries all provide hope for improvement of hearing and balance for individuals with CS. Identification of *CHD7* as the major gene involved and studies using mouse models of CS are paving the way for development of new therapies, and we are hopeful that these efforts will contribute to improving the lives of individuals with CHARGE.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest

ORCID

Donna M. Martin http://orcid.org/0000-0002-8070-2007

Yehoash Raphael http://orcid.org/0000-0003-3693-6495

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D. I. Choo, MD, is Professor and Director of Otolaryngology Head and Neck Surgery at Cincinnati Children's Hospital Medical Center (CCHMC). He also serves as co-director of the Charge Center at CCHMC and specializes in the management of ear and hearing disorders in children.

K. O. Tawfik, MD, is an Otology Fellow at the Department of Otolaryngology—Head and Neck Surgery, CCHMC. His research work and publications cover several aspects of adult and pediatric Otolaryngology including inner ear diseases and cochlear implants.

D. M. Martin, MD, PhD, is Professor of Pediatrics and Human Genetics at the University of Michigan. She evaluates and cares for individuals with CHARGE syndrome. Her areas of expertise are human genetics, mouse genetics, and developmental disorders of the nervous system.

Y. Raphael, PhD, is a Professor of Otolaryngology—Head and Neck Surgery at the University of Michigan. In his lab at Kresge Hearing Research Institute he investigates Inner Ear Biology with emphasis on prevention and cure of hearing loss and balance disorders.

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