



Review

Electrophysiology and genetic testing in the precision medicine of congenital deafness: A review

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ABSTRACT

Background: Congenital hearing loss is remarkably heterogeneous, with over 130 deafness genes and thousands of variants, making for innumerable genotype/phenotype combinations. Understanding both the pathophysiology of hearing loss and molecular site of lesion along the auditory pathway permits for significantly individualized counseling. Electrophysiologic techniques such as electrocochleography (ECochG) and electrically-evoked compound action potentials (eCAP) are being studied to localize pathology and estimate residual cochlear vs. neural health. This review describes the expanding roles of genetic and electrophysiologic evaluation in the precision medicine of congenital hearing loss.

The basics of genetic mutations in hearing loss and electrophysiologic testing (ECochG and eCAP) are reviewed, and how they complement each other in the diagnostics and prognostication of hearing outcomes. Used together, these measures improve the understanding of insults to the auditory system, allowing for individualized counseling for CI candidacy/outcomes or other habilitation strategies.

Conclusion: Despite tremendous discovery in deafness genes, the effects of individual genes on neural function remain poorly understood. Bridging the understanding between molecular genotype and neural and functional phenotype is paramount to interpreting genetic results in clinical practice. The future hearing healthcare provider must consolidate an ever-increasing amount of genetic and phenotypic information in the precision medicine of hearing loss.

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1. Introduction

The machinery of human hearing is remarkably intricate and fragile, with innumerable ways of going awry: degraded wiring (axons), malfunctioning connections (synapses), faulty pumps (ion channels) to name a few. Over 130 deafness genes are known to cause non-syndromic hearing loss (with 8000+ associated variations), an entity of substantial heterogeneity with innumerable genotype-phenotype variations (Korver et al., 2017; Marazita et al., 1993; Shearer and Hansen, 2019); moreover, over 700 syndromes are associated with hearing loss (Koffler et al., 2015). In this era of precision medicine (PM), understanding the exact pathophysiology of each individual hearing loss guides prognostication, treatment, and can predict cochlear implant (CI) outcomes—the standard auditory habilitation once hearing aids fail to provide benefit. For identifying genetic causes, next-generation sequencing has been an important boon, significantly reducing testing cost and promoting the development of lower-cost, high-yield gene panels (Rudman et al., 2018). Additionally, electrophysiological measurements can assess the health of the residual hearing apparatus and identify the site of lesion in the auditory pathway. Collectively, these tools permit noninvasive evaluation of human auditory-function-phenotypes. Combining genetic testing and electrophysiological measures characterizes this genotype-phenotype relationship, evaluates the affected sites of hearing loss, and estimates how the remaining hearing apparatus could potentially respond to hearing habilitation. The objective of this review is to describe the expanding roles of genetic and electrophysiologic evaluations in the precision medicine of congenital hearing loss.

Sensorineural hearing loss (SNHL) can be subdivided into three categories: sensory loss, central loss, and auditory neuropathy spectrum disorder (ANSD) (Korver et al., 2017). Sensory loss refers to pathologies affecting the peripheral auditory neurons whereas central losses pertain to the pathologies of the central auditory pathway. For this review, sensory losses are further partitioned based on CI physiology as detailed by Shearer and Hansen (2019). As they have described, the peripheral auditory system is further separated into the sensory partition (Organ of Corti and hair cells), synaptic partition (pre- and post-synaptic sites between hair cells and the spiral ganglion), and the neural partition (spiral ganglion and auditory nerve). ANSD is a spectrum of disorders characterized by abnormal auditory brainstem responses (ABR, suggesting neural pathology) with preserved otoacoustic emissions (OAEs, suggesting intact outer hair cell function). The site(s) of lesion for ANSD can occur anywhere from the inner hair cells (IHC), synapses, spiral ganglion, or elsewhere along the auditory nerve. An altered hearing physiology at any of these sites results in an ANSD phenotype (Eppsteiner et al., 2012; Shearer and Hansen, 2019). Identifying the site of lesion is critical for selecting optimal intervention strategies (e.g., hearing aid vs cochlear implant), providing appropriate patient counseling, and accurately predicting intervention outcomes for individual patients.

1.1. Why does knowing the genetic etiology of congenital hearing loss matter?

Approximately 70% of SNHL in congenital deafness has a genetic etiology, a number that may continue to rise as additional genetic

etiologies are discovered (Shearer et al., 2019). Recently, next generation sequencing was recommended as a first-line test for the workup of bilateral SNHL in children (Shearer et al., 2019; Shearer and Smith, 2015). Genetic testing affords a higher diagnostic yield than other assessments for SNHL such as computed tomography, magnetic resonance imaging, ophthalmology referral, renal ultrasound or electrocardiogram (Lin et al., 2011). Early genetic testing can often provide a definitive diagnosis, identify early syndromic deafness prior to the onset of other manifestations, estimate recurrence risks for family members, and potentially reduce the amount of repeat audiological testing or ancillary tests/referrals seen in SNHL workup. Moreover, a critical mass of evidence exists suggesting that identifying the molecular site of pathology can lend additional information with regards to CI outcomes (Shearer and Hansen, 2019).

1.2. How does the site of lesion affect CI outcomes?

Conventional CIs directly stimulate the auditory nerve, which is housed within the cochlea as the spiral ganglion. The CI electrode therefore “bypasses” the sensory and synaptic partitions: Organ of Corti, hair cells, and their respective synapses to the spiral ganglion (Shearer and Hansen, 2019). As such, genetic hearing losses affecting primarily these partitions have relatively favorable CI outcomes compared to other causes, and examples include mutations affecting *GJB2* (potassium homeostasis at stria vascularis) (Kikuchi et al., 1995), solute carrier family 26 member 4 (*SLC26A4*, ion transporter at stria vascularis), myosin VIIA (*MYO7A*, hair cell myosin proteins) and cadherin-23 (*CDH23*, hair cell tip links) (Nishio and Usami, 2017; Shearer and Hansen, 2019). Conversely, lesions affecting the auditory nerve such as mutations of translocation of inner mitochondrial membrane 8A (*TIMM8A*, spiral ganglion protein) (Brookes et al., 2008) or transmembrane serine protease 3 (*TMPRSS3*, unknown function but high expression in type II spiral ganglion neurons) typically have mixed to poor CI outcomes. The electrical stimulus from the implant is first encoded by the auditory nerve prior to transmission to higher auditory structures. The health of the cochlear nerve, therefore, plays an important role in post-operative CI outcomes (He et al., 2018; Incesulu and Nadol, 1998; Nadol et al., 1989; Shearer and Hansen, 2019; Shearer et al., 2018). Patients born without an auditory nerve or have pathologies with direct insult to the nerve, typically perform poorly with a CI (Nishio and Usami, 2017; Papsin, 2005).

In the case of ANSD, CI outcomes are mixed if synaptopathies are pooled together with neural partition pathologies, but results are more dependent on the exact site of lesion. Otoferlin (*OTOF*) mutations are the most common genetic etiology of ANSD, and its proteins are expressed in the pre-synaptic neurotransmitter vesicles (synaptic partition). Therefore, CI outcomes are favorable as the neural partition remains unaffected (Nishio and Usami, 2017). On the other hand, ANSD patients with concurrent cochlear nerve deficiency (CND) typically show significantly poorer outcomes than those without neural damage (Walton et al., 2008).

Recently, Shearer and Hansen (2019) and Nishio and Usami (2017) wrote extensive reviews of cochlear implant outcomes according to genetic mutation and molecular site(s) of lesion. These reviews highlight the challenges of developing an ideal study, having to account for the enormous heterogeneity of genotype/

phenotype combinations, the relative rarity of many these conditions, and that mutations within a gene can result in both syndromic and nonsyndromic hearing losses of differing inheritance. The most published studies are the commonest nonsyndromic deafness genes, whose distribution varies by ethnic cohorts: *GJB2*, *MYO7A*, *SLC26A4*, *CDH23*, *OTOF*, amongst others (Angeli et al., 2012; Nishio and Usami, 2017; Shearer and Hansen, 2019). Another important take-home from these reviews is the expanding role of the hearing healthcare provider as our understanding of the molecular physiology of hearing improves, allowing more customized counseling and care towards a patient's individual hearing loss. While CIs remain the current mainstay of treatment, the advent of targeted pharmaceutical and genetic therapies is likely in the near future (Pfungst et al., 2015b).

2. Electrophysiology to localize pathologies

The functional status of the peripheral auditory system can be evaluated using electrophysiological measures such as electrocochleography (ECoChG) (Santarelli et al., 2008; Shearer et al., 2018) and the electrically-evoked compound action potential (eCAP) (Garadat et al., 2012; He et al., 2016; Kim et al., 2010; Kirby and Middlebrooks, 2009; Long et al., 2014; Pfungst et al., 2015b). It is important to emphasize that given the peripheral nature of these measurements, the diagnostic information on the central auditory system is excluded. Due to the heavy influence of central processing on speech in noise, these peripheral measures alone cannot account for highly variable CI outcomes.

Measuring peripheral neural activity evoked from a stimulus has a longstanding history and wide-reaching uses in clinical medicine (Daube and Rubin, 2012; Erlanger and Gasser, 1924). Nerve or tissue stimulation evokes the simultaneous firing of nearby recruited nerve fibers, resulting in a compound—instead of single nerve—action potential. The conduction speed and amplitudes of these potentials are important factors to monitor, as altered potential amplitudes or delayed nerve conduction velocities signal potential disease (Parker et al., 2018). Increasing stimulus intensity typically recruits nearby fibers resulting in a large overall action potential (Waxman, 1980). The slope of action potential “growth” with increasing stimulation represents the standard growth function or Input/Output (I/O) function.

2.1. Electrocochleography and its assessment of hair cell and auditory nerve function

Electrocochleography is an acoustically-evoked technique: sound is presented to the ear and electrical potentials are measured from the cochlea and auditory nerve (Eggermont, 2017). Its clinical and research uses are broad, with selected uses such as assessing endolymphatic hydrops, measuring cochlear trauma during CI (O'Connell et al., 2017), complementing the diagnosis of ANSD, intraoperative monitoring, and estimating cochlear and neural health (Gibson, 2017). Electrical potentials can be measured in the office setting with extra-tympanic or trans-tympanic recordings, or intraoperatively via intracochlear measurements from a CI or at the round window or promontory (Gibson, 2017; Pienkowski et al., 2018). The measurement location contributes a significant role in the amplitude and shape of the recorded waveform. Specifically, intracochlear ECoChG measurements have significantly larger voltages than those recorded at the round window and are considerably larger than recordings from the tympanic membrane (Ruth et al., 1988). The four, basic overlapping ECoChG measurements include the cochlear microphonic (CM), summating potential (SP), compound action potential (CAP) and auditory nerve neurophonic (ANN) (Fig. 1) (Forgues et al., 2014; Gibson, 2017;

O'Connell et al., 2017; Zheng et al., 1997). The CM is an alternating current potential that is generated from hair cell depolarization/hyperpolarization as they transduce and respond to an acoustic stimulus (Dallos, 1986; Tasaki et al., 1952). The SP is a direct current response that reflects the average voltage difference of the hair cell membrane potential for depolarization and hyperpolarization (Dallos, 1972, 1986; Davis et al., 1958; Durrant et al., 1998). The CAP reflects the synchronous firing of auditory nerve fibers in response to acoustic stimulus onset or offset (Özdamar and Dallos, 1978; Versnel et al., 1990). The ANN represents the phase-locked response to an ongoing stimulus of auditory nerve fibers (Choudhury et al., 2012; Snyder and Schreiner, 1984). While the origins and exact contributions (e.g. hair cell vs neural) remain debated, the CM and SP are typically considered outer hair cell potentials whereas the AP and ANN are primarily neural potentials in more simplified terms (Eggermont, 2017; Shearer et al., 2018).

Over the past 10 years, there has been increasing interest in utilizing ECoChG to localize auditory pathology (Shearer and Hansen, 2019), evaluate the underlying neurophysiology in ANSD (Santarelli et al., 2009), and predict CI outcomes (Shearer et al., 2018). McMahan et al. identified two types of ECoChG waveforms recorded in patients with ANSD to help differentiate between pre- and post-synaptic lesions (McMahan et al., 2008). Riggs et al. found that those with ANSD had better hair cell potentials but worse neural survival at high frequencies using an ECoChG composite known as “nerve score” (Riggs et al., 2017). McClellan et al. (2014) showed how the composite measure known as “total response” (ECoChG-TR) can predict 40% of post-CI speech variation in adult CI users. Similarly, Fontenot et al. (2017) measured ECoChG-TRs and phonetically balanced kindergarten (PBK) word scores in 30 ANSD children and 74 non-ANSD children. They found that children with ANSD had significantly larger ECoChG-TRs than non-ANSD children. More importantly, results of regression analyses showed that the ECoChG-TR accounted for 33% and 20% of variability in PBK word scores for children with ANSD and non-ANSD children, respectively. Overall, results of these studies suggest that ECoChG may play a clinical role in localizing auditory pathology and predicting CI outcomes in varying populations.

The literature describing genetic deafness with ECoChG findings is a small but growing field, likely to expand as genetic testing becomes more prevalent. More recently, Shearer et al. (2018) utilized intracochlear ECoChG recording paradigms which bias for either hair cell or neural responses in three CI patients with *TMPRSS3* mutations. *TMPRSS3* encodes the transmembrane protease 3 protein whose function remains unclear but is found in IHCs, OHCs, and spiral ganglion neurons (Guipponi et al., 2002). The authors found that pathogenic variants in *TMPRSS3* may affect spiral ganglion function based on ECoChG measurements indicating poor neural function, which also correlated to poorer CI outcomes (Shearer et al., 2018); however, CI outcomes with *TMPRSS3* are mixed (Shearer et al., 2017).

Santarelli et al. (2015a) have reported ECoChG findings in patients with ANSD from either mutated *OTOF* or autosomal dominant optic atrophy (*OPA1* mutation) (Huang et al., 2009; Santarelli et al., 2015b). These authors routinely perform transtympanic ECoChG in the operating room (for pediatric patients) as part of their CI evaluation when ABR results may be unreliable (Santarelli et al., 2015a). Otoferlin dysfunction affects auditory nerve synapses and causes both dyssynchronous nerve transmission from altered neurotransmitter release as well as increased thresholds for neural action potential initiation (Buran et al., 2010). This manifests in pediatric *OTOF* patients with characteristically prolonged, negative ECoChG potentials. R445H missense mutations of the *OPA1* gene, a mitochondrial protein associated with numerous functions including mitochondrial membrane integrity, is

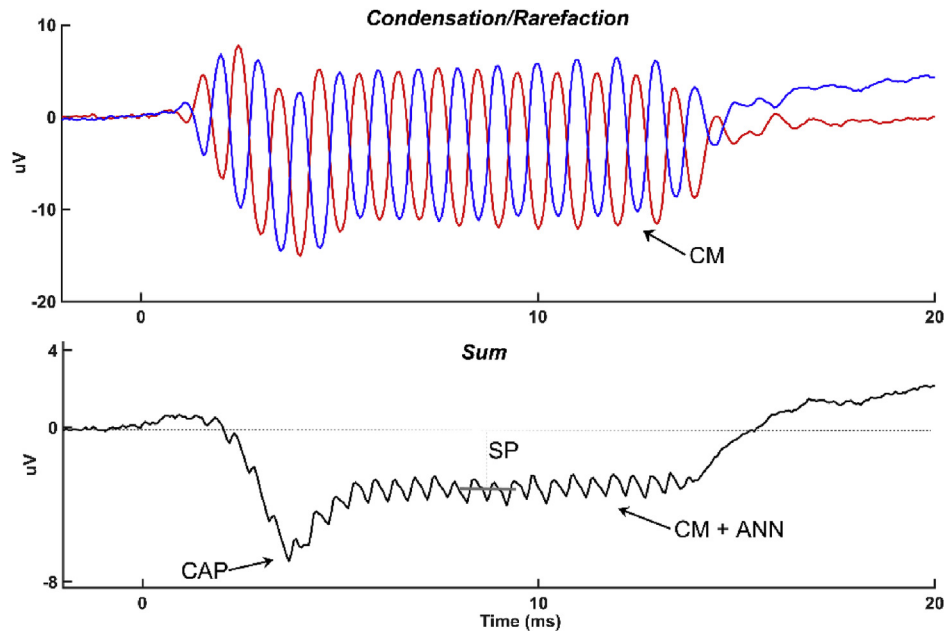


Fig. 1. An example of electrocochleography (ECoChG) evoked by a 15-ms 1 kHz tone burst. The upper panel shows the cochlear microphonic (CM) response. The CM evoked using condensation and rarefaction polarities are indicated in blue and red, respectively. The lower panel shows three different components: the summing potential (SP), the compound action potential (CAP) and the auditory nerve neurophonic response (ANN).

associated with syndromic forms of dominant optic atrophy (Yu-Wai-Man et al., 2010). *OPA1*-related hearing loss can result in preserved hair cell ECoChG potentials, but with low-amplitude prolonged negative potentials consistent with neural degeneration, localizing the primary pathology to terminal dendrites (Huang et al., 2009; Santarelli et al., 2015b).

Noguchi et al. (2004) reported ECoChG findings on 4 patients with A1555G mutations in the mitochondrial 12s rRNA gene, a common cause of hearing loss in Japan and Spain, marked by an exquisite sensitivity to aminoglycoside antibiotics. These patients had increased CM thresholds indicating cochlear hearing loss, while one patient had an elevated SP/AP ratio suggesting potential hydropic changes of unclear etiology (Noguchi et al., 2004). In a study of two Chinese patients with primarily low-frequency SNHL with *MYO7A* mutations, hydrops was not detected on ECoChG and was not considered a contributor to this low-frequency hearing loss pattern (Sun et al., 2011). Another study reported electrophysiologic findings on two pediatric patients with *GJB2* mutations with auditory neuropathy. One child showed relatively preserved OHC function with detectable CM but an undetectable CAP, SP, and ABR, suggesting pathology of the IHCs. The second child with *GJB2* and auditory neuropathy showed attenuated CAP and SPs, which suggested distal auditory nerve pathology (Santarelli et al., 2008).

2.2. The electrically-evoked compound action potential and its role in implanted patients

The electrically-evoked compound action potential in human CI users can be measured using any two intra-cochlear CI electrodes which together provide both stimulus and recording mechanisms. This is performed using the “reverse” telemetry functions found in CI programming software. In the 1990s and early 2000s, major cochlear implant manufacturers have all since developed their own proprietary version of telemetry monitoring via eCAP, allowing for electrode monitoring, device diagnostics, and device programming. The eCAP can be evoked by a biphasic (cathodic and anodic), symmetric (charge-balanced), or cathodic-leading impulse (Fig. 2).

Moreover, the phase duration, the interphase gap (IPG), and the leading stimulus polarity (e.g. cathodic-leading vs anodic-leading), can all be modulated. The eCAP responses represent a synchronized response from cochlear nerve fibers (neural partition) and is not affected by maturation of the central auditory system, meaning similar results are seen in children and adults (Eisen and Franck, 2004). Generally speaking, eCAP potentials are relatively large and robust, making it relatively resistant to contamination of myogenic activity; however, measurements can be influenced by many factors, including electrode location, stimulus intensity, stimulus polarity, etc (He et al., 2017).

The typical biphasic eCAP waveform is shown in Fig. 3 and

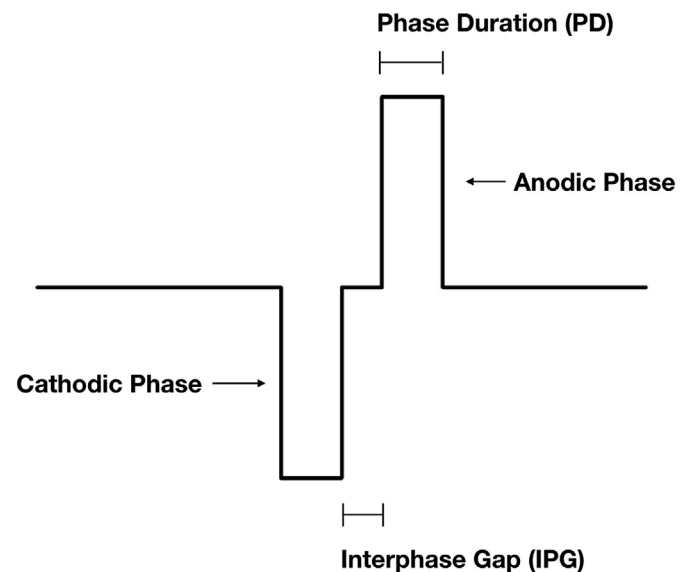


Fig. 2. A schematic illustration of a cathodic-leading, charge-balanced, biphasic pulse that can be used to evoked the eCAP.

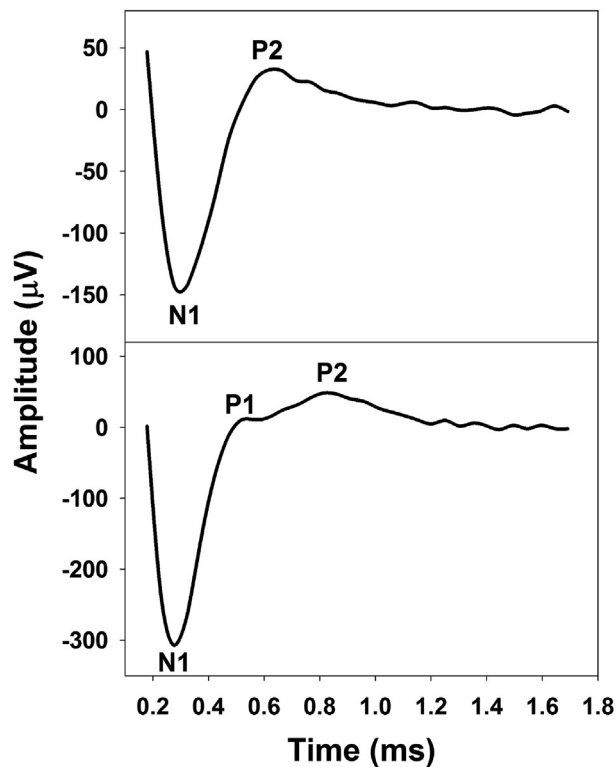


Fig. 3. Two types of electrically-evoked compound action potential (eCAP) waveforms can be recorded in cochlear implant users. Peaks are labelled for these waveforms.

typically produces 1 or 2 peaks (P1 and P2), though a single-peak waveform is much more common (Lai and Dillier, 2000). The first negative deflection is known as N1 and is predictably within 0.2–0.4 ms after stimulus onset. It is theorized that P1 represents an axonal potential, whereas P2 originates from the dendrites (Stypulkowski and Van den Honert, 1984). Due to eCAP's relatively short response latency and limitations of the recording amplifier, eCAP is subject to contamination from electrical artifacts. Therefore, removal of stimulus artifact is a critical component for accurate eCAP recordings.

A wealth of eCAP recording paradigms exist and are also reviewed extensively by He et al. (2017) ranging from relatively simple measurements for assessing neural responsiveness (single biphasic pulses) to more complicated paradigms of neural encoding and transmission of biphasic pulse trains. Those with promising correlates to neural health are discussed here, as tools for predicting neural survival has been of interest for decades. Without a means to analyze spiral ganglion neuron (SGN) counts *in vivo*, a reliable electrophysiologic surrogate is important. Temporal bone studies of SGN counts are subject to the delays between audiometric and bioelectric assessment to post-mortem histology; in that period, peri-electrode fibrosis, additional neural deterioration, and electrode migration can all occur, thereby affecting results to an unknown degree. More importantly, histologic SGN counts do not estimate how neurons respond to auditory stimulation. In comparison, eCAP recordings depend on a large group of auditory nerve fibers to fire synchronously. Therefore, results of eCAP recordings can provide information about the number and the responsiveness of surviving auditory neurons and their functional status.

The more commonly studied recording paradigm relating to neural health is the eCAP's Input/Output (I/O) function, a growth function whose slope represents how quickly the eCAP amplitude increases as the evoked stimulus intensity increases. Larger slopes

have been associated with increased SGN density in numerous animal models (Charles A Miller et al., 1994; Pflugst et al., 2015a; Pflugst et al., 2015b). Studies of human subjects have found smaller I/O slopes in those with longer durations of deafness (Schvartz-Leyzac and Pflugst, 2016) and presumably increased SGN degeneration, as well as in patients with hypoplastic auditory nerves (Nadol, 1997). Luo et al. (2020) showed larger growth function curves in implanted children with *GJB2*-related mutations compared to idiopathic deafness, suggesting better neural function in *GJB2*-related disease.

Recently, there is increased interest in how eCAP responds to changes in interphase gap (IPG) or leading pulse polarity (He et al., 2019; Hughes et al., 2018; Macherey et al., 2008; Prado-Guitierrez et al., 2006; Ramekers et al., 2014; Schvartz-Leyzac and Pflugst, 2018). Increasing the IPG results in larger slopes of the eCAP I/O function (Rattay, 1999; Rattay et al. 2001a, 2001b; Resnick et al., 2018), with larger eCAP amplitudes at fixed stimulation levels and lower eCAP thresholds (He et al., 2019; Hughes et al., 2018; Prado-Guitierrez et al., 2006; Ramekers et al., 2014). More importantly, the size of the IPG effect on the eCAP is associated with neural survival of SGN in both animal models and human CI users (He et al., 2019; Prado-Guitierrez et al., 2006; Ramekers et al., 2014). While the cathodic-leading stimulus was previously shown to better stimulate the auditory nerve in animal models (Klop et al., 2004; C. A. Miller et al., 1999), recent literature suggests in human CI users, an anodic-leading pulse is more effective (Macherey et al., 2008; Spitzer and Hughes, 2017; Undurraga et al., 2012). As such, eCAPs from anodic-leading pulses have lower thresholds, shorter latencies and larger amplitudes despite using the same stimulus level. The difference in eCAP when the stimulus changes from the cathodic-leading to the anodic-leading pulse is defined as the pulse polarity effect. It has been proposed that the size of the polarity effect provides an indicator for peripheral axon degeneration, with larger effects suggesting greater degeneration (Rattay, 1999; Rattay et al. 2001a, 2001b; Resnick et al., 2018). Results of studies in human CI users are consistent with this idea (Carlyon et al., 2018). Overall, these studies suggest that eCAP sensitivity to the pulse polarity and the IPG effect can potentially be used to estimate auditory neural health.

Lastly, measurements of the auditory nerve absolute refractory period (ARP) and relative refractory period (RRP) can also provide information on neural health and are measurements of temporal responsiveness. Following depolarization from a stimulus, all peripheral nerves have an ARP (unable to be depolarized) followed by an RRP (depolarization only with sufficiently strong stimulus). ARP and RRP can be estimated using the eCAP refractory recovery function (RRF) (Charles A Miller et al., 2001). Longer ARPs are also associated with reduced nerve densities in animal models (Shepherd et al., 2004). He et al. (2018) have shown that implanted children with CND typically have longer ARPs than those with normal caliber cochlear nerves.

3. Summary and future directions

Despite the tremendous progress in discovering new deafness genes, the effects of individual genes on auditory neural function remain poorly understood. Bridging the understanding between molecular, neural, and functional phenotypes is paramount to interpreting an ever-increasing amount of genetic information available in clinical practice. In this article, we have reviewed the basics of genetic hearing loss as it pertains to CI outcomes, auditory electrophysiologic tests (ECochG and eCAP), and how they complement each other in the advanced diagnostics and prognostication of hearing habilitation. Used together, these measures improve the understanding of pathophysiological insults to the auditory

system, allowing for improved counseling for CI candidacy/outcomes or other habilitation strategies. In general, genetic information and electrophysiologic information can both help identify site of lesions within the auditory pathway; knowing the site of lesion and the health of the auditory nerve can predict CI outcomes in greater precision. The future hearing healthcare provider must therefore be able to consolidate an increasing amount of genetics and phenotypic information available in the precision medicine of hearing loss.

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Author contributions

KZ, SH and XZL conceptualized the overall topic together. KZ drafted the initial version of this paper, provided critical comments, drafted and approved the final version of this paper. SH provided critical comments, drafted and approved the final version of this paper. XZL provided critical comments and approved the final version of this paper. OFA, AE, WJR, SMP and DY provided critical comments and approved the final version of this paper.

Declaration of competing interest

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