INTRODUCTION

Age-related hearing loss (ARHL) is a complex neurodegenerative disease and a major challenge in the field of auditory neuroscience. Worldwide projections indicate 432 million adults are affected by disabling hearing loss (defined as thresholds >40 dB HL in the better hearing ear averaged across 0.5, 1, 2, and 4 kHz; WHO, 2018). When these data are dichotomized by sex the prevalence of disabling hearing loss is greater in men compared to women, equating to 242 million men and 190 million women worldwide. Recent outputs from the Global Burden of Disease rank hearing loss as the fourth leading cause of years lived with a disability and reinforce its status as a growing economic burden (Wilson, Tucci, Tucci, Merson, & O'Donoghue, 2017). For a disability often described as invisible, loss of hearing has a devastating effect on quality-of-life. It presents a tremendous communication barrier and often leads to social isolation and loneliness (Davis et al., 2016). In recent years links between ARHL and an increased risk of cognitive decline, dementia, and depression have shifted the focus to identifying shared molecular mechanisms that may be driving these common neurodegenerative pathologies (Hardy et al., 2016; Loughrey, Kelly, Kelly, Kelley, Brennan, & Lawlor, 2018; Shen et al., 2018). This has enormous therapeutic potential as it raises the concept of future overlapping strategies for their treatment and prevention.
ARHL is a multifactorial disease governed by both genetic and environmental factors (noise, ototoxic drugs), and modulated by the interaction between them (Bowl & Dawson, 2019). Heritability estimates for ARHL lie between 35% and 70% (Christensen, Frederiksen, Frederiksen, & Hoffman, 2001; Gates, Couropmitree, Couropmitree, & Myers, 1999; Hendrickx et al., 2013; Raynor et al., 2009) and noise exposure is a major environmental component that is difficult to separate from the underlying genetic basis (Liberman, 2017; Van Eyken, Van Camp, & Van Laer, 2007). Studies in aged animals and human temporal bones have given great insight into the histological deficit with degenerative changes in the sensory hair cells, the spiral ganglion neurons, and the stria vascularis predominantly described; Figure 1 (Keithley, 2019; Ohlemiller, 2004; Schuknecht, 1964; Schuknecht & Gacek, 1993). In the last two decades tremendous progress has been made in identifying the genes responsible for early onset congenital hearing loss and describing their role in normal cochlear function (Bowl & Brown, 2018; Ingham et al., 2019; Steel & Kros, 2001). In comparison, our understanding of the genetic risk factors and the molecular pathways they modulate in ARHL has been slower.

**FIGURE 1** Schematic of the human inner ear to show the main types of degeneration in the aging cochlea (sensory, strial, and neural) that are predominantly described in the literature. (a) The inner ear is composed of the membranous labyrinth and consists of the vestibular system (for balance) and the cochlea (for hearing). Distinct sensory, strial (also known as metabolic), and neural forms of ARHL were first proposed by Schuknecht (1964). However, it is widely accepted that the etiology of ARHL is due to a combination of these pathologies and can involve any cochlear structure. (b) A cross-section through a single coil of the cochlear spiral. Degenerative changes in the stria vascularis, which is responsible for maintaining the endocochlear potential that is essential for hearing, underlie strial forms of ARHL. Loss of the auditory nerve fibers underlie neural ARHL. sm, scala media; st, scala tympani; sv, scala vestibuli. (c) Schematic of the organ of Corti (boxed region in b). Loss of the sensory hair cells, the mechanotransducers of the cochlea, underlie sensory ARHL. In recent years attention has focused on the loss of the synaptic connections between inner hair cells and afferent nerve fibers (synaptopathy) which is thought to precede sensory hair cell loss. is, inner sulcus; tc, tunnel of Corti.

**Significance**

Compared to the progress in understanding which genes underlie early onset childhood deafness, we still do not fully understand which genetic risk loci and molecular pathways contribute to age-related hearing loss. Here I review the evidence why it is important to consider sex as a biological variable in understanding this complex, neurodegenerative pathology in women and in men.
1.1 | The impact of sex as a biological variable in human disease

Historically basic and preclinical research studies have largely ignored the impact of sex as a biological variable (SABV). Information on the sex of the animals/participants included in the studies was either not clarified, or studies were conducted on just one sex, typically male, and research outcomes assumed to translate to both sexes (Beery & Zucker, 2011; Shansky, 2019). Many studies on ARHL have not escaped this sex bias despite the fact it has long been known that ARHL, like many other complex traits, exhibits sex differences in prevalence (Corso, 1963; Cruickshanks et al., 1998; Helzner et al., 2005), severity (Jerger, Chmiel, Chmiel, Stach, & Spretnjak, 1993; Pearson et al., 1995), and age at onset (Corso, 1963; Davis, 1995; Pearson et al., 1995).

In a recent study Villavisanis, Schrode, and Lauer (2018) reported that of 231 original research articles on ARHL over a 10-year period (2006–2015), one third did not disclose the sex of the animals used in the study. Of those that did report sex, only half used both sexes and one third used males only (Villavisanis et al., 2018). A similar preference for only using male animals in experiments was identified in research studies conducted on noise-induced hearing loss (NIHL) over the 5-year period between 2011 and 2015 (Lauer & Schrode, 2017).

To combat sex bias in basic and preclinical research, the U.S. National Institutes of Health (NIH) set out a new mandate for the inclusion of SABV in scientific research in 2016 (Clayton, 2016, 2018; Clayton & Collins, 2014). Many journals, including the Journal of Neuroscience Research, followed suit (Prager, 2017). Following the NIH mandate more attention has been focused in recent years on the role of SABV in health and disease (Shansky & Woolley, 2016). Recent reviews have illustrated the importance of understanding SABV in the etiology of complex disease including: neurodegenerative diseases (Honarpisheh & McCullough, 2019; Pinares-Garcia, Stratikopoulos, Stratikopoulos, Zagato, Loke, & Lee, 2018; Ullah et al., 2019), cardiovascular disease (De Bellis et al. 2019), inflammation and hypertension (Sylvester & Brooks, 2019), and immune system function and autoimmune conditions (Gubbels Bupp, Potluri, Potluri, Fink, & Klein, 2018). Elucidating the role that SABV plays in complex disease is vital for understanding the underlying pathophysiology and improving therapeutic approaches. This review will examine our understanding of auditory function and ARHL in the context of SABV. For the purpose of this review the term sex is used rather than gender. In humans, biological sex is defined by the specific combination of the sex chromosomes (XX for females and XY for males), whereas the term gender may not always pertain to the sex chromosomes present from birth (Khramtssova, Davis, Davis, & Stranger, 2019). Further, although sex differences in hearing are reported for both the central and peripheral auditory system, this review will primarily focus on peripheral ARHL.

2 | BIOLOGICAL SEX MODULATES LOSS OF AUDITORY FUNCTION WITH AGING

It has been known for decades that ARHL is more common (Corso, 1963; Cruickshanks et al., 1998; Helzner et al., 2005) and more severe (Jerger et al., 1993; Pearson et al., 1995) in men compared to women. In men, deterioration in auditory thresholds can be detected from the second and third decade of life, but women often do not show symptoms until several years (Corso, 1963; Pearson et al., 1995), or decades later (Davis, 1995).

Differences in noise exposure between men and women have long been proposed as the precipitating factor underlying sex differences in the prevalence and age at onset of ARHL. In one of the first large longitudinal studies to incorporate audiometric data, the Framingham Heart Study Cohort, the prevalence of ARHL was more common in men compared to women. However, since information on noise exposure history was not recorded, interpretation of these results was difficult (Gates, Cooper, Cooper, Kannel, & Miller, 1990). Later epidemiological studies on ARHL that factored noise exposure history into the study design showed that sex differences in susceptibility to ARHL cannot just be attributed to differences in noise exposure (Cruickshanks et al., 1998; Girotto et al., 2011a; Pearson et al., 1995). For example, the Epidemiology of Hearing Loss Study (Beaver Dam, Wisconsin) showed that men were more than four times as likely to have ARHL compared to women (OR = 4.42, 95% CI = 3.73–5.24). This difference remained significant even after adjusting for lifestyle covariates including noise exposure (OR = 3.65, 95% CI = 2.97–4.49) (Cruickshanks et al., 1998).

The effect of SABV on hearing function is not limited to differences in the prevalence and age at onset of ARHL. It has also long been known that there are differences in hearing sensitivity between men and women. More than half a century ago Corso (1963) performed one of the first large audiometric studies designed to investigate the effect of age and biological sex on pure-tone hearing thresholds (0.25–8 kHz), in a population that reported minimal levels of industrial and recreational noise. Both sexes exhibited a progressive loss of high-frequency hearing that extended to the low-frequencies with aging, which is the consensus that is accepted today. However, women displayed better high-frequency hearing sensitivity (above 3 kHz) compared to men at most ages. Conversely, men had better low-frequency (0.25, 0.5 kHz) hearing compared to women (Corso, 1963).

The effect of SABV on hearing sensitivity across the lifespan in participants with no evidence of excessive noise exposure is well-documented. Jerger et al. (1993) recapitulated these results using audiometric data from 28,688 individuals enrolled in U.S. and European National Health Surveys (Jerger et al., 1993). Similar sex differences in low- and high-frequency hearing sensitivity with aging were reported in the Baltimore Longitudinal Study of Aging (Pearson et al., 1995) and the Beaver Dam Epidemiology of Hearing Loss Study (Cruickshanks et al., 1998). These early audiological studies suggest
that the 1-2 kHz region of the cochlea is a pivotal frequency at which minimal effect of SABV on hearing sensitivity is detected. Beyond this pivotal region women have better high-frequency hearing sensitivity than men (toward the cochlear base) and men have better low-frequency hearing sensitivity than women (toward the cochlear apex; Corso, 1963; Cruickshanks et al., 1998; Jerger et al., 1993; Pearson et al., 1995).

Sex differences in the prevalence, severity, and age at onset of ARHL continue to prevail in recent epidemiological studies. Hoffman, Dobie, Losonczy, Themann, and Flamme (2017) compared audiometric data from different cycles of the U.S. National Health and Nutrition Examination Survey (1999-2004 vs. 2011-2012). Pure-tone thresholds were obtained on 3,831 individuals and pure-tone averages (PTAs) calculated across low (PTA_{speech frequency}) and high (PTA_{high frequency}) frequencies. Hearing impairment was defined by a PTA >25 dB HL. Under these definitions, male sex remained a significant risk factor for ARHL, particularly in the high frequencies (PTA_{high frequency} male vs. female): OR = 3.8, 95% CI = 2.7-5.4 vs. PTA_{speech frequency}; male vs. female: OR = 1.8, 95% CI = 1.1-3.0; data adjusted for lifestyle covariates including noise exposure; Hoffman et al., 2017). In comparison, in a recent prospective cohort study on members of the Dutch population from Rotterdam, sex differences in ARHL showed some signs of narrowing (Homans et al., 2017). The average difference in hearing loss (defined by a PTA across 0.5-4 kHz) between men and women was less when compared with earlier studies. However, when the pure-tone frequencies were examined independently significant sex differences in hearing sensitivity were detected consistent with the trends described earlier (Homans et al., 2017). These lifetime sex differences in hearing sensitivity between men and women range from small to moderate, up to 16 dB according to recent data (see Figure 2; Homans et al., 2017).

Comparing sex differences in the prevalence, age at onset, and severity of ARHL across different cohort studies is difficult. The age of the participants often varies widely and recent cohort studies incorporate more stringent definitions of hearing loss compared to earlier studies; greater than 35 and/or 40 dB HL in the better hearing ear (Homans et al., 2017) versus greater than 25 dB HL in the worse hearing ear (Cruickshanks et al., 1998). Further, a limitation of some of these cohorts is that the participants are predominantly over 50 years of age (Cruickshanks et al., 1998; Gates et al., 1990; Homans et al., 2017). It is widely accepted that premenopausal women are protected from the onset of a declining auditory system compared to age-matched men. In healthy middle-aged women, the age they enter the menopausal transition as opposed to age per se coincides with a relatively rapid deterioration in hearing (Hederstierna, Hultcrantz, Hultcrantz, Collins, & Rosenhall, 2010; Svedbrant, Bark, Bark, Hultcrantz, & Hederstierna, 2015). Accelerated sensorineural hearing loss is also a well-characterized feature of women with Turner’s syndrome who are estrogen deficient (Beckman, Conway, Conway, & Cadge, 2004; Bonnard, Bark, Bark, & Hederstierna, 2019). Consequently, if hearing in young adults of each sex is not included in the study design, valuable information on the impact of sex hormones on the maintenance of auditory function with aging will not be captured.

Sex differences in hearing are not just a feature of ARHL, but they are present throughout life. The biological basis of these differences is currently unclear, and in order to understand these mechanisms it is important to understand how these changes present at birth as well as during life.

3 | SEX DIFFERENCES IN AUDITORY PHYSIOLOGY FROM BIRTH

3.1 | Otoacoustic emissions (OAEs)

OAEs, a measure of outer hair cell (OHC) function, are routinely used in newborn hearing screens. Discovered by Kemp in 1978 (Kemp, 1978) they are a bio-acoustical consequence of somatic electromotility; OHCs contract synchronously and modulate their length in response to sound-induced vibrations. This process leads to “cochlear amplification”; movement of the OHCs amplifies the traveling sound wave along the cochlea, augmenting hearing sensitivity. It is also essential for the high selectivity of tonotopic frequency discrimination in hearing (Kemp, 2002). OAEs arise when some of the mechanical energy generated through OHC electromotility is dissipated away from the organ of Corti toward the tympanic membrane (eardrum) in the form of vibrations through the middle ear. Such vibrations can be detected acoustically in the ear canal and provide a quick, noninvasive means to assess OHC function. OAEs are recorded as transient-evoked OAES (TEOAEs) in response to an acoustic stimulus, or distortion product OAEs (DPOAEs) in response to two adjacent pure-tone stimuli (lower and higher) presented simultaneously. Spontaneous OAEs can also arise in the form of sustained oscillations due to internal feedback of the OHC vibrations (Kemp, 2002).

It has long been known that sex differences in OAEs are present in human neonates from birth (Cassidy & Ditty, 2001; Gordts, Naessens, Naessens, Mudde, & Clement, 2000; Kei, McPherson, McPherson, Smyth, Latham, & Loscher, 1997; McFadden, 1998; Saitoh et al., 2006; Strickland, Burns, Burns, & Tubis, 1985) and become more apparent as the stimulus frequency increases (Cassidy & Ditty, 2001). Female newborns present stronger TEOAEs that exhibit higher signal-to-noise ratios (Kei et al., 1997; Saitoh et al., 2006) and higher response amplitudes (Cassidy & Ditty, 2001; Saitoh et al., 2006) compared to those from newborn males. Spontaneous OAEs are also more prevalent and stronger in female newborns compared to males (Qi, Cheng, Cheng, En, Huang, & Zhang, 2014; Strickland et al., 1985); a trend which remains in adulthood (Snihur & Hampson, 2011).

In the largest study of TEOAE recordings to date, TEOAEs were examined in 30,000 newborns over a 6-year period from 1998 (Berninger, 2007). The median TEOAE level was compared as a function of frequency; the magnitude of the response was significantly greater for female newborns compared to males, particularly...
FIGURE 2  Pure-tone air conduction thresholds in men versus women with aging. These data were obtained from the Rotterdam study; charts were constructed from a tabulated version of these data (Table 1) originally reported by Homans et al., (2017). (a) Mean hearing thresholds from the right ear are shown at frequencies between 0.25 and 8 kHz in 5-year age groups; error bars: SEM. *Mean hearing thresholds were significantly better compared to the other sex at the specified frequency ($p < 0.05$). (b) The first three charts in A have been reproduced in the boxed region with a shorter scale on the $y$-axis (40–50 dB HL maximum) to show the sex differences in hearing sensitivity with greater clarity. The sex-specific differences in hearing sensitivity with aging observed here are consistent with the trends described in the literature in earlier studies. Women have a better high-frequency hearing compared to men throughout life. Conversely, men have a better low-frequency hearing compared to women.
in the 3–4 kHz frequency range (Berninger, 2007). Similar results have been reported using DPOAE recordings. In a study of 185 neonates, tested 4 days after birth, the mean amplitude of the DPOAE at 4 kHz was higher in females compared to males (Gordts et al., 2000). However, some studies using DPOAE recordings report a much smaller effect size of SABV compared to those conducted with TEOAEs (McFadden, Martin, Martin, Stagner, & Maloney, 2009). It has been suggested that differences in the stimuli used to generate DPOAEs compared to TEOAEs could underlie this discrepancy (McFadden et al., 2009). Alternatively, it has been proposed that the underlying biological mechanism responsible for sex differences in OAEs has less impact on DPOAEs (McFadden et al., 2009). One biological mechanism that has long been proposed to explain sex differences in OAEs is the prenatal-androgen-exposure hypothesis. The high level of androge hormones expressed in-utero during sexual development in males is thought to impact the mechanics responsible for cochlear amplification such that weaker OAE emissions are generated in males (McFadden, 2009; McFadden et al., 2009).

With aging OAE strength declines in humans and it is recognized that OAEs are more prominent in newborns and young infants compared to adults (Abdala & Dhar, 2012). In females, decline in OAE strength has been linked with oral contraceptive use (McFadden, 2000; Snihur & Hampson, 2012a), and the menopause in both humans (Karaer & Tuncay, 2019) and animals (Guimaraes, Zhu, Zhu, Cannon, Kim, & Frisina, 2004). In young mice on a CBA background minimal sex differences in DPOAE strength are observed. However by middle age, DPOAE amplitudes in female mice are significantly larger compared to males and diminish only once the female mice reach old age (Guimaraes et al., 2004). Since mice generally undergo menopause between 12 and 14 months of age, a protective effect of estrogen is thought to explain the delayed decline in DPOAE strength in the female mice compared to males.

TEOAEs and spontaneous OAEs also fluctuate in women during the menstrual cycle (Bell, 1992; Haggerty, Lusted, Lusted, & Morton, 1993) with the highest signal intensity detected in the late follicular phase when endogenous estrogen levels are highest (Al-Mana, Ceramic, Ceramic, Djananbakch, & Luxon, 2010). TEOAE strength is also correlated with seasonal changes in circulating testosterone levels in rhesus monkeys (McFadden, Pasanen, Pasanen, Raper, Lange, & Wallen, 2006) and humans. In male monkeys, weaker TEOAEs are detected during the breeding season (fall/autumn) when sex hormone levels are highest (McFadden et al., 2006).

These studies suggest that from birth subtle differences in OHC electrophysiology are present between the sexes. OHCs in males appear to be less physiologically active and more vulnerable to the aging process compared to females.

3.2 | Auditory brainstem response (ABR) recordings

The ABR is an auditory-evoked potential which in humans is commonly measured with scalp electrodes. It is characterized by a series of waveforms, I–VII in humans (Jewett, Romano, Romano, & Williston, 1970) and I–V in mice (Willott, 2006). In humans and most mammals, it is generally agreed that wave I is a postsynaptic response manifesting from the VIII cochlear nerve. The origins of the subsequent waves are ambiguous, but they are thought to originate from ascending regions along the auditory pathway in the brainstem (Jewett et al., 1970; Parkkonen, Fujiki, Fujiki, & Makela, 2009; Willott, 2006).

In humans, it has been known for decades that SABV modulates the output (waveform) of the ABR (Jerger & Hall, 1980; Lopez-Escamez, Salguero, Salguero, & Salinero, 1999; McFadden, 1998; Michalewski, Thompson, Thompson, Patterson, Bowman, & Litzelman, 1980; Sturzebecher & Werbs, 1987). Female newborns exhibit greater peak amplitudes, shorter ABR latencies, and shorter inter-peak intervals compared to males, particularly when the ABRs are recorded from the right ear (Maurizi et al., 1988; Sining, Cone-Wesson, Cone-Wesson, & Abdala, 1998). These sex differences in the ABR predominantly reside within waves III and V, and the inter-peak intervals I–III and I–V, suggesting sex differences in central auditory processing. The correlation of ABR wave III and V latencies with the menstrual cycle in women supports this hypothesis; longer peak III and V latencies are detected when the levels of circulating estrogen are highest (Elkind-Hirsch, Wallace, Wallace, Malinak, & Jerger, 1994; Yadav, Tandon, Tandon, & Vaney, 2002). The impact of SABV on central auditory processing warrants further attention but is beyond the scope of this review. A recent review on the topic is lacking in the literature and would be most valuable.

Reductions in ABR wave I amplitude in the presence of a normal audiogram are a hallmark of cochlear synaptopathy. This neuropathy occurs when afferent synaptic connections between IHCs and auditory nerve fibers are lost. This loss is not detected by standard clinical measures of recording hearing thresholds, but is considered an early pathologic feature of both NIHL and ARHL (Kujawa & Liberman, 2009, 2015; Liberman, 2017; Sergeyenko, Lall, Lall, Liberman, & Kujawa, 2013; Wu et al., 2019). The impact of SABV on synaptopathy is not fully understood. In one recent study, a trend for smaller ABR wave I amplitudes in normal-hearing adults that reported the greatest history of noise exposure was reported in response to suprathreshold clicks and 4kHz tone bursts (Stamper & Johnson, 2015a). However, the omission of SABV in the study design resulted in valuable information on the impact of SABV on ABR wave I being lost (Stamper & Johnson, 2015a). When these results were later stratified by SABV this trend was only observed in the female participants (Stamper & Johnson, 2015b). It is possible these results simply reflect the sex differences in the sample size (20 females vs. 10 males) and the study was underpowered in males. However, examination of the cohort by SABV revealed that, on average, females had larger wave I amplitudes compared to males and the extent of the noise exposure was skewed in favor of the males making interpretation of the results difficult (Stamper & Johnson, 2015b).

In mice, various studies report sex differences in auditory thresholds with aging. Mice on the C57BL/6J background are predisposed to an early onset hearing loss due to a genetic variant in the Cdh23 gene at the Ahr locus (Johnson, Erway, Erway, Cook, Willott,
We have primarily understood the function of estrogen as a sex hormone. However, estrogen is a multifaceted hormone whose mode of action is governed by a complicated network of signaling pathways. Estrogen is a lipid-soluble hormone synthesized from cholesterol in a series of consecutive steps that end with the conversion of testosterone into estrogen by the enzyme aromatase. In females, estrogen is mainly produced by the ovaries, but it can also be synthesized locally in extragonadal tissues in both sexes. In premenopausal women, the most abundant form of estrogen is 17β-estradiol, with serum levels ranging from 0.11 to 2.20 nM according to the stage of the menstrual cycle. Postmenopause 17β-estradiol levels decline in women and are more comparable to those reported for men; 0.04 versus 0.10 nM, respectively (Vrtacnik et al., 2014). Alternatively, the estrogen-ER complex can bind a different transcription factor that in turn binds their cognate site to regulate gene transcription (indirect genomic mechanism). In the ligand-independent mechanism, ERs are activated through phosphorylation by intracellular second messengers (Cui et al., 2013; Marino et al., 2006; Vrtacnik et al., 2014). Estrogen can also elicit ultra-rapid functional changes in a cell type-specific manner that are too fast to be explained by gene transcription and subsequent protein translation. This non-genomic mode of estrogen signaling is mediated by membrane-associated truncated forms of the ERs, including G protein-coupled estrogen receptor-1 (GPER1; GPR30). This mode of signaling results in the activation of various intracellular signal transduction cascades including: MAPK/ERK, phosphatidylinositol 3 kinase/AKT, phospholipase C/protein kinase C, and cAMP/protein kinase A (Cui et al., 2013; Marino et al., 2006; Srivastava & Evans, 2013; Vrtacnik et al., 2014). Interestingly, all of these intracellular signal transduction cascades are associated with cochlear function (Jagger & Ashmore, 1999; Kurioka et al., 2015; Park & Kalinec, 2015; Sha, Chen, Chen, & Schacht, 2010), but the effect of SABV on these pathways in the cochlea is largely unknown.

In the brain, there is much evidence that estrogen has a role in neuroprotection (Arevalo, Azcoitia, Azcoitia, & Garcia-Segura, 2015; Azcoitia, Barreto, Barreto, & Garcia-Segura, 2019; Cui, Shen, Shen, & Li, 2013; Genazzani, Pluchino, Pluchino, Luisi, & Luisi, 2007). Given this neuroprotective role, it is perhaps unsurprising that there is a wealth of literature that estrogen, the classical estrogen receptors (ERs), and the estrogen-related receptors (ERRs) play a critical role in maintaining auditory function (Charitidi, Meltser, Meltsers, Tahera, & Canlon, 2009; Delhez, Lefebvre, Lefebvre, Pequeux, Malgrange, & Delacroix, 2020; Hultcrantz, Simonoska, Simonoska, & Stenberg, 2006; Shuster, Depireux, Depireux, Mong, & Hertzano, 2019; Williamson, Zhu, Zhu, Pinerio, Ding, & Frisina, 2020). Despite this, our understanding of the modes of action of estrogen in the cochlea are far from clear.

4.1.1 | Mechanisms of estrogen signaling

The mechanisms by which estrogen signaling is relayed are well described in the literature. Yet, we know little regarding the molecular targets of these pathways in the cochlea. Estrogen signaling is relayed through both nuclear and membrane receptors. The classical nuclear receptors ER-alpha (ERα; ESR1; NR3A1) and ER-beta (ERβ; ESR2; NR3A2) function as transcription factors and can be activated in a ligand-dependent or ligand-independent manner. In ligand-dependent signaling estrogen (the ligand) binds to ERα and/or ERβ and elicits a conformational change in the ER. This promotes dissociation of the ER from chaperones in the cytosol, dimerization, and activation of the transcriptional domain. Upon dimerization, the estrogen-ER complex translocates to the nucleus and binds to estrogen response elements within target gene promoters to directly regulate gene expression (known as the direct genomic mechanism; Marino, Galluzzo, Galluzzo, & Ascenzi, 2006, Cui et al., 2013, Vrtacnik et al., 2014). Alternatively, the estrogen-ER complex can bind a different transcription factor that in turn binds their cognate site to regulate gene transcription (indirect genomic mechanism). In the ligand-independent mechanism, ERs are activated through phosphorylation by intracellular second messengers (Cui et al., 2013; Marino et al., 2006; Vrtacnik et al., 2014). Estrogen can also elicit ultra-rapid functional changes in a cell type-specific manner that are too fast to be explained by gene transcription and subsequent protein translation. This non-genomic mode of estrogen signaling is mediated by membrane-associated truncated forms of the ERs, including G protein-coupled estrogen receptor-1 (GPER1; GPR30). This mode of signaling results in the activation of various intracellular signal transduction cascades including: MAPK/ERK, phosphatidylinositol 3 kinase/AKT, phospholipase C/protein kinase C, and cAMP/protein kinase A (Cui et al., 2013; Marino et al., 2006; Srivastava & Evans, 2013; Vrtacnik et al., 2014). Interestingly, all of these intracellular signal transduction cascades are associated with cochlear function (Jagger & Ashmore, 1999; Kurioka et al., 2015; Park & Kalinec, 2015; Sha, Chen, Chen, & Schacht, 2010), but the effect of SABV on these pathways in the cochlea is largely unknown.

4.1.2 | Classical estrogen receptors (ERs) in the cochlea

Our understanding of the expression of the classical ERs (ERα and ERβ) in the inner ear predominantly comes from studies in
rodents (Charitidi, Meltser, Meltsers, & Canlon, 2012; Meltser et al., 2008; Motohashi et al., 2010; Stenberg, Wang, Wang, Sahlin, & Hultcrantz, 1999) with limited data available on expression in human cochlear tissue (Stenberg et al., 2001). Expression of ERs is generally considered the more widespread of the two. In mice, ERα has been detected in the sensory hair cells, supporting cell populations of the organ of Corti, the spiral ganglion neurons, the stria vascularis, Reissner’s membrane, and the spiral ligament. However, there is some ambiguity in the literature over where these two ERs are expressed with studies in different mouse strains reporting conflicting results (Charitidi et al., 2012; Meltser et al., 2008; Motohashi et al., 2010; Stenberg et al., 1999). The expression of the ERs in the cochlea also appears to be modulated by SABV. One study in CBA/J mice reported stronger immunoreactivity to ERα in many cochlear sub-regions of young female mice compared to males. Since the spatial expression pattern of ERα did not appear to differ between male and female mice in this study, this suggests that SABV could affect the relative abundance of ER expression levels in the cochlea (Motohashi et al., 2010). Further, animal studies show that expression of ERs in the auditory system are sensitive to hormonal fluctuations during the estrous cycle and in CBA/Ca mice, ERα is negatively correlated with the level of circulating estrogen (Charitidi et al., 2012). Cyclic fluctuations in auditory function are also well-documented in women during the menstrual cycle with the greatest hearing sensitivity around the time of ovulation when levels of circulating estrogen are highest (Al-Mana et al., 2010; Souza et al., 2017; Swanson & Dengerink, 1988). It is also important to consider that both ERα and ERβ undergo alternative splicing. Various studies describe the presence of truncated isoforms of the ERs that can form homo- and heterodimers with each other and with full-length ERs to modulate ER activity (Charitidi et al., 2009; Jia, Dahlman-Wright, Dahlman-Wright, & Gustafsson, 2015; Vrtačnik et al., 2014). Yet we have little knowledge which isoforms of the ERs are expressed in the cochlea and whether SABV impacts their expression.

The first direct evidence that the loss of ERβ protein impairs auditory function comes from studies with mice carrying a germline knockout for the ERβ gene (Meltser et al., 2008; Simonoska et al., 2009). Meltser et al. (2008) compared ABR thresholds in mice lacking: (a) Erα, (b) Erβ, and (c) Cyp19a1. Cyp19a1 encodes the enzyme aromatase responsible for the conversion of androgens into estrogen, the primary source of estrogen in extragonadal tissues in both sexes. In young mice no effect on auditory sensitivity was observed in any of the three knockout models. However, both Erβ and Cyp19α1 knockout mice were more sensitive to the effect of acoustic trauma compared to wild-type mice (Meltser et al., 2008). No effect of SABV was observed in this study. However, pretreatment of wild-type and Cyp19α1 knockout mice with a selective agonist of Erβ conferred protection from the acoustic trauma, which the authors postulated may be mediated by brain-derived neurotrophic factor (Meltser et al., 2008). In a separate study, Simonoska et al. showed that Erβ knockout mice are deaf by 12 months of age and exhibit a greater degeneration of the organ of Corti compared to wild-type mice (Simonoska et al., 2009). However, the effect of SABV on loss of ERβ function in the cochlea with aging is not known as the sex of the animals was not described in this study (Simonoska et al., 2009).

Deletions of other genes involved in estrogen signaling also show auditory deficits: Lrp2 (Konig et al., 2008) and Wbp2 (Buniello et al., 2016). Mice carrying a germline knockout for the Lrp2 gene, which encodes megalin (an endocytic receptor that binds lipophilic metabolites, including estrogen) exhibit a progressive hearing loss from 3 months of age (Konig et al., 2008). In the cochlea, expression of megalin overlaps with ERβ in the marginal cells of the stria vasularis. The megalin knockout mice exhibit a reduction in microvilli in the apical membrane of strial marginal cells, more abundant lipofuscin granules, and impaired uptake of FITC-labeled β-estradiol into strial marginal cells. These observations support a role for estrogen in the function of the stria marginal cells (Konig et al., 2008). It is plausible this may be mediated through an inhibitory mechanism on potassium channel gating (Lee & Marcus, 2001). WBP2 encodes a transcriptional coactivator for ERα. Loss of Wbp2 underlies progressive high-frequency hearing loss in mice and mutations in the WBP2 gene are associated with early onset hearing loss in humans (Buniello et al., 2016). In Wbp2-deficient mice expression of both Erα and Erβ is reduced, and ABR and DPOAE thresholds are elevated from 4 weeks of age concurrent with a reduction in the amplitude of ABR wave l. Swelling of the IHC afferent terminals and a range of postsynaptic defects characteristic of glutamate excitotoxicity were also observed, providing indirect evidence for a role of the ERs in IHC synaptic function (Buniello et al., 2016).

### 4.1.3 Nonclassical estrogen-related receptors (ERRs) in the cochlea

The nonclassical ERs, estrogen-related receptor α (ESRRA; NR3B1), β (ESRBB; NR3B2), and γ (ESRRG; NR3B3) form the NR3B subgroup of the nuclear receptor superfamily. They share a high degree of structural homology with the well-characterized classical ERs, but they are orphan nuclear receptors. Hence, regulation of their downstream target genes is not ligand-dependent and they are regarded constitutively active transcription factors. However, their potency as transcriptional activators is modulated in a cell-context manner by interaction with specific coregulator proteins (Eichner & Giguere, 2011; Huss, Garbacz, Garbacz, & Xie, 2015; Tremblay & Giguere, 2007).

ERRs are expressed at elevated levels in tissues that are subject to a high metabolic demand, such as the heart, skeletal muscle, and kidney. Here they play a crucial role in regulating energy metabolism (Alaynick et al., 2007, 2010; Dufour et al., 2007; Rangwala et al., 2010). There appears to be a degree of interplay between the ERRs and the classical ERs for binding site occupancy in estrogen responsive genes. In addition to binding their cognate site (the estrogen-related receptor element) the ERRs are also capable of binding the classical estrogen response element in specific gene promoters (Eichner & Giguere, 2011; Huss et al., 2015; Tremblay & Giguere, 2007).
Evidence that the ERRs are important for auditory function was first identified in mice with a targeted deletion of Esrb in the inner ear (Chen & Nathans, 2007). Esrb conditional knockout mice are deaf by 3 months of age, endolymph production is impaired, and the expression of ion channel and transporter genes in strial marginal cells is abnormal (Chen & Nathans, 2007). The significance of the ESRRB gene in hearing is also confirmed in humans. Collin et al. (2008) were the first to report that mutations in the ESRRB gene underlie autosomal recessive, non-syndromic hearing loss DFNB35 in a large consanguineous family of Turkish origin and in three large families from Pakistan (Collin et al., 2008). Subsequently an array of studies reported that rare variants in ESRRB (predominantly in the ligand-binding domain) underlie DFNB35 in families from Pakistan (Khan et al., 2019; Lee et al., 2011), Tunisia (Ben Said et al., 2011), and the Czech Republic (Safta Brozkova et al., 2012). Recently Bhatt et al. (2016) reported that individuals heterozygous for a non-synonymous single nucleotide polymorphism (SNP) rs61742642, in the ligand-binding domain of ESRRB, are more susceptible to mild noise exposure compared to non-carriers (Bhatt et al., 2016). However, the effect of biological sex was not examined in this study. It is not known if men that carry this SNP are more susceptible to noise exposure compared to women.

Our own studies have demonstrated the first direct link between ESRRG and auditory function in both humans and mice (Nolan, Maier, et al., 2013). In a Genome-Wide Association Study (GWAS) performed on a cross-section of the U.K. population at age 44–45 years (the 1958 British Birth Cohort), we identified an association between genetic variation in the ESRRG gene and adult hearing function. SNP rs2818964 at the ESRRG locus was associated with poorer hearing thresholds at 4 kHz, albeit under the cutoff for genome-wide significance. However, when we followed these findings up in two independent genetic cohorts, we found that the minor allele of ESRRG SNP rs2818964 is associated with ARHL in women, but not in men (Nolan, Maier, et al., 2013). First, an ARHL case–control cohort from London U.K., the strongest evidence of association was identified in women with a family history of ARHL. Second, a cohort from isolated populations of Italy and Silk Road Countries, quantitative measures of hearing function showed that the association was strongest in the low-mid frequencies. Unlike the women in the 1958 British Birth Cohort, the women from both the London and the Silk Road cohorts were predominantly of postmenopausal age. Hence, we postulated that the decline in inherent estrogen levels with menopause may be a key factor underlying the association of ESRRG with risk of hearing loss in women (Nolan, Maier, et al., 2013). In the same study we showed that auditory function is impaired in mice carrying a germline knockout for the Esrg gene strengthening the evidence for a role of ESRRG in hearing (Nolan, Maier, et al., 2013). More recently Schilit et al. (2016) demonstrated that a translocation between chromosomes 1 and 5 that disrupts the ESRRG gene is strongly implicated as the cause of congenital hearing loss and mild developmental delay in a young female patient (Schilit et al., 2016).

ESRRG is not the only gene that exhibits a sex-specific association with ARHL. In an early candidate-gene study to identify genetic risk loci for ARHL, Van Eyken et al. (2006) reported that two SNPs in the sensory hair cell potassium voltage-gated channel, KQT-like subfamily, member 4 (KCNQ4) gene are associated with high-frequency ARHL in women, but not in men (Van Eyken et al., 2006). Further, our own early studies using a candidate-gene approach have implicated a functional SNP in the mitochondrial superoxide dismutase 2 (SOD2) gene promoter is associated with ARHL in men, but not in women (Nolan, Cadge, Cadge, Gomez-Dorado, & Dawson, 2013).

### 4.2 Sex differences in genetic architecture

To date the GWAS on ARHL do not explain the heritability estimates for ARHL in the literature. Early studies did not reach genome-wide significance, but they have revealed a wealth of important candidate genes associated with ARHL and/or hearing function (Friedman et al., 2009; Fransen et al., 2015; Girotto et al., 2011b; Huyghe et al., 2008, Van Laer et al., 2010). Many explanations have been proposed for this so-called missing heritability in ARHL, including genetic variants that are rare in the population that each contribute a small effect on ARHL risk, the definition used to define the phenotype and the size of the population under study (Bowl & Dawson, 2019; Wells, Newman, & Williams, 2019). All these factors impact the power of the GWAS to detect a significant genetic association. Recent studies that have sought to increase sample size by meta-analysis (Hoffmann et al., 2016; Nagtegaal et al., 2019; Vuckovic et al., 2015; Wolber et al., 2014), or by using extremely large cohorts (Wells, Freidin, et al. 2019) have yielded more significant genome-wide loci. However, our understanding of sex differences in the genetic architecture driving ARHL is largely unknown.

There are three distinct genetic models that are hypothesized to explain sex differences in the epidemiological parameters of complex traits: (a) The Carter Effect/Sex-Dependent Liability Thresholds; (b) Sex Chromosome Effects, and (c) Gene-By-Environment Interactions. They will not all be discussed in detail here. Instead, the reader is referred to the recent excellent review on the topic by Khramtsova et al. (2019). It is likely that all three models contribute to the phenotype of a complex trait, like ARHL. Notably, in the Carter effect model one sex requires a greater number of risk alleles compared to the other (known as a greater genetic liability) in order to exhibit the disease phenotype. In this model the heritability of the trait is higher in the sex with the lower prevalence (Khramtsova et al., 2019). Therefore, if the Carter effect is applied to females, siblings of female probands are more likely than siblings of male probands to be affected themselves. This concept ties in with the early observations made on the heritability of ARHL by Gates et al. (1999).

Gates compared PTAs (PTA\textsubscript{Low}, PTA\textsubscript{Medium}, PTA\textsubscript{High}) as a measure of hearing function between genetically unrelated people (spouse pairs) and genetically related people (parent–child pairs; sibling pairs). As expected, hearing thresholds did not correlate in spouses.
However, they did correlate in mother–daughter and mother–son pairs, but not in father–child pairs (Gates et al., 1999) supporting a stronger heritability of the trait in women compared to men.

Currently, the majority of the GWAS on ARHL and/or normal hearing function have been performed on a sex-combined dataset and various statistical approaches have been used to adjust the data for an effect of SABV. Few GWAS for hearing have performed sex-stratified analysis where men and women are dichotomized into distinct subgroups. Consequently, it is important to consider that if the genetic architecture for ARHL differs between men and women, performing a GWAS on a sex-combined dataset may reduce the power of the study. This raises the possibility that the genetic variants that modulate ARHL risk specifically in women or men could run the risk of remaining undiscovered (Khramtsova et al., 2019; Ober, Loisel, Loisel, & Gilad, 2008; Rawlik, Canela-Xandri, Canela-Xandri, & Tenesa, 2016).

4.3 | Sex differences in the cochlear transcriptome

The endogenous level of estrogen (and other sex steroid hormones) over the lifespan could modulate the cochlear transcriptome within specific cochlear cell types by multiple signaling mechanisms as discussed in Section 4.1.1. Therefore, it is important to consider that the expression level of the genes that are important for the maintenance of normal cochlear function may differ between men and women throughout life. Sex differences are also reported for various epigenetic mechanisms that govern gene regulation including DNA methylation patterns, microRNA gene silencing, and histone modifications. The latter results in sex differences in the chromatin that is available for gene transcription (Ling, Sugathan, Sugathan, Mazor, Fraenkel, & Waxman, 2010; Liu, Morgan, Morgan, Hutchison, & Calhoun, 2010). To add to the complexity, there exists a feedback loop whereby estrogen signaling can impact the epigenetic modes of gene regulation and these epigenetic mechanisms can in turn regulate the expression of the ERs (Vrtacnik et al., 2014). The causal genetic variants that underlie complex disease risk are regarded by many to impact gene regulation as opposed to protein structure and function (Gallagher & Chen-Plotkin, 2018). Consequently, a future challenge will be to understand the impact of estrogen signaling on the gene regulatory framework in specific cochlear cell types and whether this interacts with functional risk loci for ARHL.

4.4 | Sex differences in cochlear structure and function

The hypothesis that estrogen could modulate cochlear physiology is not new. A greater amount of myelination and an increased fiber diameter of the auditory nerve fibers, increased synaptic efficiency, and a more rapid hormonal-related neuronal conduction in females compared to males have all been postulated since the late 1970s (Maurizi et al., 1988). Differences in the extent of the efferent inhibitory medial olivocochlear fibers due to sex differences in prenatal androgen exposure has also been extensively discussed in the literature by McFadden and proposed to underlie the greater hearing sensitivity and larger OAEs in women compared to men (McFadden, 1993, 2009).

At present we do not know if the functional differences in auditory sensitivity between women and men can be correlated with anatomical differences in cochlear structure. Early studies on aged human temporal bones report a progressive loss of outer hair cells manifesting from the apical cochlear coil, not just the base (Wright, Davis, Davis, Bredberg, Ulehlova, & Spencer, 1987). A finding which has been confirmed in a more recent study (Wu et al., 2019). However, our understanding of the effect of SABV on OHC loss dissected by frequency is limited. In humans and mammals the type-I afferent nerve fibers that form synaptic contacts with the inner hair cells are most abundant in the middle of the cochlea (Liberman, 2017; Wu et al., 2019). In humans this correlates with the 1 kHz region of the cochlea and is the frequency at which human hearing is regarded the most sensitive (Liberman, 2017; Wu et al., 2019). Interestingly, 1 kHz is also the pivotal region of the cochlea at which hearing sensitivity does not appear to differ between men and women throughout life. However, it is not yet known if the density of these nerve fibers exhibit sex differences in humans at specific frequencies along the tonotopic map of the cochlea. In insect ears (mosquitos), there are sex-specific differences in the spatial distribution of the efferent auditory pathway that is responsible for modulating auditory sensitivity (Su, Andres, Andres, Boyd-Gibbins, Somers, & Albert, 2018). Sex-specific differences in intra-swarm acoustic communication in mosquitoes also exist for the mechanotransduction channel; the gating properties of this channel differ between the sexes with male mosquitos having more sensitive transducers compared to females (Su et al., 2018). Whether such a huge phylogenetic leap could be made to the mechanotransduction channel in mammals and humans is unknown.

5 | A UNIFYING HYPOTHESIS

SABV modulates susceptibility to ARHL with differences in the prevalence, severity, and age of onset between men and women widely documented in the literature. Numerous studies from both humans and animals show that SABV impacts cochlear function. Differences in the output of OAE and ABR recordings exist between men and women from birth. Further, when the audiogram in humans is dissected by frequency along the tonotopic axis of the cochlea and stratified by sex, loss of auditory sensitivity over the lifespan shows evidence of a sex-specific effect that is frequency dependent.

In other species, including birds (Krentzel & Remage-Healey, 2015), fish (Fergus, Feng, Feng, & Bass, 2015), anuran amphibians (frogs/toads) (Shen et al., 2011), and mosquitoes (Su et al., 2018), sex-specific differences in auditory sensitivity are coupled to reproduction and linked to the acoustic communication essential for courtship behavior (see also the excellent review by
CONFLICT OF INTEREST
Lisa S. Nolan is a subject associate editor for the Journal of Neuroscience Research. Lisa S. Nolan: Conceptualization; Writing - Original Draft Preparation; Writing - Review and Editing.

ORCID
Lisa S. Nolan https://orcid.org/0000-0002-9972-0723

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6 | FUTURE PERSPECTIVES
A concerted effort is required to understand the biological mechanisms whereby SABV impacts cochlear function. The inaccessibility of the inner ear in humans presents a tremendous barrier to any study which seeks to understand the impact of SABV on the transcriptome within different cochlear cell types essential for hearing function, and it makes a transcriptome-wide association study (Wainberg et al., 2019) on ARHL a major challenge, if not impossible. However, we can use animal models to dissect the modes of estrogen signaling in the cochlea and use these to gain insight into the consequence of these signaling pathways on normal cochlear function in women and men. It is widely recognized that SABV influences both the pharmacodynamics and pharmacokinetics of various drugs. Consequently, it will be important that any pharmacological therapeutic approach for ARHL does not adopt a “one-size fits all” strategy, but instead focuses on precision medicine at the molecular level tailored to the individual and differentiated by sex. By understanding the role SABV plays in the etiology of complex disease like ARHL, ability to understand and manipulate the underlying molecular pathways driving ARHL will be dramatically increased.

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(Caras, 2013). Even in humans we can draw parallels between reproduction and auditory function because auditory sensitivity in women fluctuates during the menstrual cycle, with an enhanced auditory sensitivity coinciding with the time of ovulation. Therefore, it is plausible that any sex-specific differences in auditory physiology in humans are simply a by-product of our evolution. They are an evolutionary relic that at one point may have been essential for our survival and reproduction.

Therefore, it is tempting to hypothesize that the molecular pathways that are important for sex-specific modulation of the auditory system during mating behavior in nonhumans, and are conserved across species, are the ones that will manifest in humans in sex-specific susceptibility to complex disease like ARHL. In fish, there is already some evidence to support this concept (Fergus et al., 2015). Genes that are correlated with auditory tuning during mating in a teleost fish, the midshipman fish, are enriched in metabolic pathways involving ion channels, neurotransmission, and steroid signaling. Two of these genes are members of the ERR family, ESRRB and ESRRG (Fergus et al., 2015). The former (ESRRB) is a gene already linked with susceptibility to noise exposure in humans (Bhatt et al., 2016), and the latter (ESRRG) is a gene already linked with susceptibility to ARHL in women (Nolan, Maier, et al., 2013).

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.

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