REVIEW ARTICLE

Development and evolution of the vestibular apparatuses of the inner ear

Kacey Mackowetzky[1] | Kevin H. Yoon[1] | Emily J. Mackowetzky[1] | Andrew J. Waskiewicz[1,2]

[1]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

[2]Women & Children's Health Research Institute, University of Alberta, Edmonton, Alberta, Canada

Correspondence
Andrew J. Waskiewicz, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.
Email: aw@ualberta.ca

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Abstract

The vertebrate inner ear is a labyrinthine sensory organ responsible for perceiving sound and body motion. While a great deal of research has been invested in understanding the auditory system, a growing body of work has begun to delineate the complex developmental program behind the apparatuses of the inner ear involved with vestibular function. These animal studies have helped identify genes involved in inner ear development and model syndromes known to include vestibular dysfunction, paving the way for generating treatments for people suffering from these disorders. This review will provide an overview of known inner ear anatomy and function and summarize the exciting discoveries behind inner ear development and the evolution of its vestibular apparatuses.

KEYWORDS
balance, inner ear, otocysts, otoliths, saccule, semi-circular canals, signaling pathways, utricle, vertigo, vestibular apparatus, vestibular system, vestibulocochlear nerve

1 | INTRODUCTION

Our sensory organs provide us with a way to interact with the world by detecting chemical or physical stimuli, either internally or within our environment, and transducing these signals along a neural circuit to the brain for processing and response determination (Straka, 2020; Straka et al., 2016). The inner ear is one of the most fascinating sensory organs because it is responsible for two key systems of perception: the auditory system for perceiving sound and the vestibular system for sensing balance and spatial orientation (Brister et al., 2020; Straka, 2020). Research into both systems is essential for understanding the diseases that impact inner ear function, but as 1–2 of every 1,000 children born in the United States suffer from a hearing impairment and roughly 2 billion older adults suffer from hearing loss, this has attracted a greater deal of research into the auditory system (Centers for Disease Control & Prevention, 2017; Hoffman et al., 2017; Schilder et al., 2019; Yamoah et al., 2020). However, the necessity for research into the vestibular system remains, given that approximately 3.3 million (5.2%) children between the ages of 2 and 17 in the United States

suffer from some form of vestibular dysfunction, as characterized by delayed motor development or chronic dizziness and balance issues (Li et al., 2016). Head and neck injuries resulting in lesions to the inner ear and its internal circuit to the brain and congenital disorders whose symptoms do not present until later in life can also result in vestibular dysfunction, usually in the form of bouts of vertigo, the illusion of spinning or tilting, either of the environment or oneself (Gurr & Moffat, 2001; Kolev & Sergeeva, 2016; Yamoah et al., 2020). This illusory perception of motion confers physical risk, although sensory impairments associated with the inner ear also have an impact on mental health. Notably, disabilities related to inner ear dysfunction, particularly those that are acute, also contribute to a higher incidence of anxiety and depression (Yuan et al., 2015). Therefore, to combat health-related issues caused by vestibular disorders, research into the development of the inner ear is essential for treating patients suffering from inner ear dysfunction and identifying preventative methods for birth defects associated with the vestibular system.

A growing body of work invested in comprehending the complex developmental program behind the vestibular apparatuses of

the inner ear has provided an extensive foundation for understanding diseases involved in this system (Fattal et al., 2018; Toriello & Smith, 2013). In fact, recent research in animal models has already helped to identify the genetic factors behind some disorders that include vestibular dysfunction, such as Branchio-oto-renal syndrome, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Delpire-McNeill syndrome, and Usher syndrome (Table 1; Ceruti et al., 2002; Kulkarni et al., 2012; McNeill et al., 2020; Stinckens et al., 2001; Weil et al., 1996). Therefore, the purpose of this review is to give an overview of what is known about inner ear anatomy and function and to summarize the exciting research that has already been conducted on the development and evolution of this intricate sensory organ.

2 | ANATOMY AND FUNCTION OF THE INNER EAR VESTIBULAR SYSTEM

The inner ear is a complex, labyrinthine structure situated within the temporal bone on each side of the head (Figure 1; Fekete & Wu, 2002a; Fritzsch et al., 2002). It is composed of a central vestibule connected to the cochlea on one side, which houses the auditory machinery of the ear, and three orthogonally situated semi-circular canals on the other. A series of mosaic patches—composed of mechano-receptive hair cells and their support cells housed within the central vestibule and the semi-circular canals—represent the vestibular sensory organs of the ear.

The first of these vestibular organs, the semi-circular canals, each terminate in a sac that houses an epithelium composed of hair cells and support cells that are sheathed by a gelatinous mass (Mescher, 2010). This gelatinous mass, known as cupula, extends from each sensory epithelia to the ampulla, a bulge situated directly across from an epithelium within a given canal. The hair cells within this epithelium are covered by stereocilia, mechanoreceptors that, when deflected, trigger the release of a chemical transmitter to the nerve terminals synapsed to their base (Barrett et al., 2012; Elliott et al., 2018). The deflection of these stereociliary bundles in the semi-circular canals is triggered in a unique way during angular, or "rotational," accelerations of the head. When the head turns, the inner ear moves with it, but the endolymph, a potassium-rich fluid contained within the ear, remains inert. Because this fluid remains stationary, it exerts force against the cupula, deflecting the stereocilia within to sense the speed of rotation relative to the axis of the canal.

The second group of vestibular organs is known as the maculae, two sensory epithelia positioned such that one, the saccule, is oriented vertically within the vestibule, while the other, the utricle, is oriented horizontally (Chagnaud et al., 2017; Purves et al., 2001). As such, they detect motion in the vertical and horizontal planes, respectively, although their anatomy and function differ from that of the semi-circular canals. Instead of detecting angular rotations, these sensory organs are responsible for detecting linear accelerations and the position of the head relative to gravity. Additionally, although the hair cells within the maculae are similarly covered by a heavy, gelatinous mass, it is embedded with small, calcium carbonate

crystals known as otoliths or otoconia. This otolithic membrane moves according to the direction of gravity, such that when the head is tilted, the membrane shifts, deflecting the stereocilia of the underlying hair cells (Hain & Helminski, 2007).

Another important, although non-sensory, component of the vestibular system is the endolymphatic duct, which extends dorsally from the utricular and saccular ducts housed within the central vestibule and through the vestibular aqueduct to connect with the endolymphatic sac, which is partially contained between the bony labyrinth surrounding the ear and the posterior cranial fossa (Kopecky et al., 2013; Lo et al., 1997). While endolymph is produced primarily by the dark cells of the vestibular labyrinth and stria vascularis of the cochlea, this endolymphatic duct and sac system is primarily responsible for regulating inner ear pressure by either decreasing it through the resorption of endolymph or by increasing it through the production of proteoglycans to attract and bind water (Kingma & van de Berg, 2016; Wackym et al., 1987).

3 | OVERVIEW OF DEVELOPMENT OF THE VESTIBULAR STRUCTURES OF INNER EAR

While vestibular disorders can arise from physical trauma to the head or neck (Kolev & Sergeeva, 2016), they can also occur from the improper development of the vestibular structures previously described. As such, understanding the developmental program of the inner ear and identifying the genes involved in this process can be informative for elucidating the mechanisms of inner ear dysfunction, whether they appear at birth or manifest later in life (Fattal et al., 2018). This in turn allows for the identification of potential targets for gene therapy and the development of chemical therapeutic agents for preventative strategies and treatment of vestibular defects.

The aim of the following sections is to discuss some of the most important discoveries already made in animal models concerning the developmental program of the inner ear. It will outline the first step of inner ear development, the induction of the otic placode, which is the source of all inner ear cell types, and the subsequent formation of the otic vesicle, a closed-off portion of the neural ectoderm that is subjected to signaling cues that pattern, or confer an identity on, the various otic cell types that will eventually arise in this sensory organ (Figure 2). These sections will also provide a summary description of how the vestibular structures are thereby formed, highlighting genes that have also been identified in human patients suffering from vestibular disorders, some of which can be found in Table 1.

4 | THE OTIC PLACODE AND FORMATION OF THE OTIC VESICLE

Important discoveries have already been made concerning the developmental plan of the inner ear leading up to the induction of an

TABLE 1 Genes associated with vestibular dysfunction in human syndromes or disease phenotypes, as well as their phenotypes in animal disease models. OMIM reference numbers have been included where available

Human gene name	Possible human behavioral or anatomical phenotypes	Animal model gene name and disease phenotypes	Citations
CADHERIN 23; CDH23 (605516)	- Delayed motor skills associated and vestibular dysfunction - Associated with Usher syndrome, type 1D (601067) and typed 1D/F digenic (601067); deafness, autosomal recessive 12 (601386); and Pituitary adenoma 5, multiple types (617540)	- Cdh23 (mouse): hyperactivity, head-tossing, and circling behavior; disorganized stereociliary bundles - cdh23 (zebrafish): balance defects and circling swimming behavior; disorganized stereociliary bundles	- Bolz et al., 2001) - Di Palma et al. (2001) - Holme and Steel (2002) - Smith, Lee, al., (1992) - Söllner et al. (2004) - Wada et al. (2001) - Zheng et al. (2004)
CHLORIDE INTRACELLULAR CHANNEL 5; CLIC5 (607293)	- Vestibular dysfunction later in life - Associated with deafness, autosomal recessive 103 (616042)	- Clic5 (mouse): impaired balance; reduced number and increased degeneration of hair cells; abnormal or missing stereocilia	- Gagnon et al. (2006) - Seco et al. (2015)
CHROMODOMAIN HELICASE DNA-BINDING PROTEIN 7; CHD7 (608892)	- Semi-circular canal hypoplasia or agenesis - Vestibulocochlear nerve hypoplasia - Associated with CHARGE syndrome (214800) and Hypogonadotropic hypogonadism 5 with or without anosmia (612370)	- Chd7 (mouse): head shaking and circling; truncation or size reduction of the posterior and lateral semi-circular canals; improper fusion within the central pouch prior to canal formation	- Bosman et al. (2005) - Jongmans et al. (2008) - Van de Laar et al. (2007) - Vissers et al. (2004)
COAGULATION FACTOR C HOMOLOGY; COCH (603196)	- Variable vestibular dysfunction - Caused by mucopolysaccharide depositions within the inner ear, resulting in the degeneration of dendritic fibers - Associated with deafness, autosomal recessive 110 (618094) and autosomal dominant 9 (601369)	- Coch (mouse): vestibular dysfunction (as determined through analysis of vestibular-evoked potential)	- Jones et al. (2011) - Khetarpal et al. (1991) - Manolis et al. (1996) - Robertson et al. (2006)
ELONGATION FACTOR Tu GTP-BINDING DOMAIN-CONTAINING 2; EFTUD2 (603892)	- Agenesis of lateral canals - Associated with mandibulofacial dysostosis, Guin-Almedia type (MFDGA) (610536)		- Gordon et al. (2012)
EPHRIN RECEPTOR EPHA2; EPHA2 (176946)	- Enlarged vestibular aqueduct - Associated with Pendred syndrome (274600) and Cataract 6, multiple types (116600)		- Li, Nishio, et al. (2020)
EPHRIN RECEPTOR EPHB2; EPHB2 (600997)	- Enlarged vestibular aqueduct - Associated with Pendred syndrome (274600); Bleeding disorder, platelet-type, 22 (618462); and prostate cancer/brain cancer susceptibility, somatic (603688)	- Ephb2 (mouse): abnormal otoconia; absence of the endolymphatic duct; dysplasia of endolymphatic fluid space (particularly of the semi-circular canals); mis-localized endolymphatic sac ion-transport cells	- Cowan et al. (2000) - Li, Nishio, et al. (2020) - Raft et al. (2014)
EPITHELIAL SPLICING REGULATORY PROTEIN 1; ESRP1 (612959)	- Hypoplasia of the lateral semi-circular canal (deficiency restricted to the central bony island) - Associated with deafness, autosomal recessive 109 (618013)	- Esrp1 (mouse): common crus and lateral semi-circular canal dysgenesis; low incidence of central cyst without vestibular outgrowths	- Rohacek et al. (2017)

(Continues)

TABLE 1 (Continued)

Human gene name	Possible human behavioral or anatomical phenotypes	Animal model gene name and disease phenotypes	Citations
EYA TRANSCRIPTION COACTIVATOR AND PHOSPHATASE 1; EYA1 (601653)	- Enlarged vestibule; enlarged vestibular aqueduct; lateral semi-circular canal dysplasia - Associated with otofaciocervical syndrome (166780); anterior segment anomalies with or without cataract (602588); Branchio-otic syndrome 1 (602588); and Branchio-oto-renal syndrome 1 with or without cataracts (113650)	- *Eya1* (mouse): inner ear agenesis; truncated endolymphatic duct, truncated anterior or posterior ampullae and canals; absent semi-circular canals; small or missing maculae; reduced number of hair cells - *eya1* (zebrafish): imbalanced and circling swimming behavior; reduced response to auditory/vibrational stimuli; small otoliths, absent hair cells in cristae; reduced number of hair cells in maculae	- Ceruti et al. (2002) - Kozlowski et al. (2005) - Namba et al. (2001) - Song et al. (2013) - Whitfield et al. (1996) - Xu et al. (1999) - Zou et al. (2008)
FIBROBLAST GROWTH FACTOR 3; FGF3 (164950)	- Delayed gross motor skills in infancy due to balance impairment - Inner ear agenesis (Michel aplasia) or hypoplasia (cystic vestibulum) - Absence of the vestibulocochlear nerve - Associated with deafness, congenital with inner ear agenesis, microtia, and microdontia (610706)	- *Fgf3* (mouse): inner ear agenesis - *fgf3* (zebrafish): inner ear agenesis	- Alsmadi et al. (2009) - Sensi et al. (2011) - Tekin et al. (2007) - Tekin et al. (2008) - Wright and Mansour (2003)
FIBROBLAST GROWTH FACTOR RECEPTOR 2; FGFR2 (176943)	- Semi-circular canal hypoplasia - Associated with Apert syndrome (101200); Antley-Bixler syndrome without genital anomalies or disordered steroidogenesis (207410); - Beare–Stevenson cutis gyrata syndrome (123790); Bent bone dysplasia syndrome (614592); Craniofacial-skeletal-dermatologic dysplasia (101600); Craniosynostosis, non-specific (N.A.); Crouzon syndrome (123500); Gastric cancer, somatic (613659); Jackson-Weiss syndrome (123150); LADD syndrome (149730); Pfeiffer syndrome (101600); Saethre-Chotzen syndrome (101400); Scaphocephaly and Axenfeld-Rieger anomaly (N.A.); and Scaphocephaly, maxillary retrusion, and mental retardation (609579)	- *Fgfr-2* (mouse; IIIb isoform): disrupted morphogenesis: dysgenesis of membranous labyrinth, resulting in cystic cavities; rudimentary vestibulocochlear ganglion; and sensory patches	- Park et al. (1995) - Quintero-Rivera et al. (2006) - Wang et al. (2002)
GAP JUNCTION PROTEIN, BETA-2; GJB2 (121011)	- Vertigo - Associated with Bart-Pumphrey syndrome (149200); Deafness, autosomal dominant 3A (601544) and autosomal recessive 1A (220290); Hystrix-like ichthyosis with deafness (602540); Keratitis-ichthyosis-deafness syndrome (148210); Karetoderma, palmoplantar with deafness (148350); and Vohwinkel syndrome (124500)	- *cx30.3* (zebrafish): reduced ear size	- Chang-Chien et al. (2014) - Dodson et al. (2011)

TABLE 1 (Continued)

Human gene name	Possible human behavioral or anatomical phenotypes	Animal model gene name and disease phenotypes	Citations
GAP JUNCTION PROTEIN, BETA-6; GJB6 (604418)	- Vertigo - Associated with deafness, autosomal dominant 3B (612643), autosomal recessive 1B (612645), and digenic GJB2/GJB6 (220290); also associated with Ectodermal dysplasia 2, Clouston type (129500)		- Dodson et al. (2011)
H6 FAMILY HOMEOBOX 2; HMX2 (600647)	- Unsteady, wide-based gait, and delayed walking in children - Mondini malformations: enlarged vestibule and endolymphatic duct (N.A)	- *Hmx2* (mouse): failed enlargement of the pars superior and semi-circular canal aplasia - *hmx2* (zebrafish): larval circling, looping, and spiraling swimming behavior; mono-otolith (fused otoliths) situated over saccular macula; reduced number of utricular hair cells	- Feng and Xu (2010) - Miller et al. (2009) - Wang et al. (2001) - Wang et al. (2004)
H6 FAMILY HOMEOBOX 3; HMX3 (613380)	- Unsteady, wide-based gait, and delayed walking - Mondini malformations: enlarged vestibule and endolymphatic duct (N.A.)	- *Hmx3* (mouse): loss of hair cells; absence of lateral semi-circular duct crista - *hmx3* (zebrafish): larval circling, looping, and spiraling swimming behavior; mono-otolith (fused otoliths) situated over saccular macula; reduced number of utricular hair cells	- Feng and Xu (2010) - Miller et al. (2009) - Wang et al. (2004)
HOMEOBOX A1; HOXA1 (142955)	- Semi-circular canal aplasia; common cavity - Associated with Athabaskin brainstem dysgenesis syndrome (601536) and Bosley–Salih–Alorainy syndrome (601536)	- *Hoxa1* (mouse): variable malformations, including dilated membranous labyrinth, incomplete periotic capsule ventromedially, and missing semi-circular canals	- Lufkin et al. (1991) - Pasqualetti et al. (2001) - Tischfield et al. (2005)
HOMEOBOX A2; HOXA2 (604685)	- Inner ear agenesis - Associated with Microtia, hearing impairment, and cleft palate (AR) (612290) and Microtia with or without hearing impairment (AD) (612290)		- Alasti et al. (2008)
JAGGED 1; JAG1 (601920)	- Vestibular pathology, as characterized with disequilibrium when walking - Hypoplasia of the posterior canal and aplasia of the anterior canal - Associated with Deafness, congenital heart defects, and embryotoxon (617992); Alagille syndrome 1 (118450); and Tetralogy of Fallot (187500)	- *Jag1* (mouse): heading-shaking/weaving behavior and poor negative geotaxis (vertical climbing ability); missing ampullae	- Le Caignec et al. (2002) - Tsai et al. (2001)
LIM HOMEOBOX TRANSCRIPTION FACTOR 1, ALPHA; LMX1A (600298)	- Vertigo - Associated with deafness, autosomal dominant 7 (601412)	- *Lmx1a* (mouse): impaired righting reflex, circling behavior, hyperactivity, and ataxic gate	- Bergstrom et al. (1999) - Millonig et al. (2000) - Wesdorp et al. (2018)
LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 2; LRP2 (600073)	- Malformation of the semi-circular canal and vestibule - Associated with Donnai-Barrow syndrome (222448)		- Kantarci et al. (2007)

(Continues)

TABLE 1 (Continued)

Human gene name	Possible human behavioral or anatomical phenotypes	Animal model gene name and disease phenotypes	Citations
LYSINE-SPECIFIC METHYLTRANSFERASE 2D; KMT2D (602113)	- Malformations associated with Mondini dysplasia: variable aplasia, hypoplasia, or dysplasia of the semi-circular canals and an enlarged endolymphatic sac and duct - Associated with Kabuki syndrome 1 (147920)	- *Kmt2d* (mouse): difficulty maintaining balance and gait abnormalities	- Igawa et al. (2000) - Yamamoto et al. (2019)
MYOSIN, HEAVY CHAIN 9, NONMUSCLE; MYH9 (160775)	- Scheibe dysplasia: cochleosaccular degeneration (CSD) - Associated with deafness, autosomal dominant 17 (603622) and macrothrombocytopenia and granulocyte inclusions with or without nephritis or sensorineural hearing loss (155100)		- Lalwani et al. (1997) - Lalwani et al. (1999)
MYOSIN VIIA; MYO7A (276903)	- Vestibular dysfunction - Associated with Usher syndrome type 1B (276900) and deafness, autosomal dominant 11 (601317) and autosomal recessive 2 (600060)	- *Myo7a* (mouse): splayed/disorganized stereociliary bundles; hyperactivity, head-shaking, and circling behaviors - *myo7aa* (zebrafish): splayed/disorganized stereociliary bundles; reduced response to auditory/vibrational stimuli and looping or corkscrewing swimming patterns	- Ernest et al. (2000) - Gibson et al. (1995) - Holme and Steel (2002) - Nguyen et al. (2015) - Weil et al. (1996)
OTOGELIN; OTOG (604487)	- Delayed motor development; persistent vestibular hyporeflexia - Associated with deafness, autosomal recessive 18B (614945)	- *Otog* (mouse): Abnormal head and body posture, abnormal landing pattern (commonly land on back, and circular underwater swimming behavior; detached otoconia and ampullary cupula)	- Schraders et al. (2012) - Simmler, Cohen-Salmon, et al. (2000)
PAIRED BOX 2; PAX2 (167409)	- Enlarged chamber of the inner ear - Associated with Papillorenal ("Renal-coloboma") syndrome (120330) and Glomerulosclerosis, focal segmental, 7 (616002)	- *Pax2* (mouse): enlarged central vestibule; absent utricle, saccule, and ampullae	- Eccles and Schimmenti (1999) - Favor et al. (1996) - Sanyanusin et al. (1995)
POTASSIUM CHANNEL, VOLTAGE-GATED, KQT-LIKE SUBUNIT SUBFAMILY, MEMBER 1; KCNQ1 (607542)	- Gross motor developmental delay and impaired balance - Associated with Jervell and Lange-Nielsen syndrome (220400); Long QT syndrome 1 (195200); Short QT syndrome 2 (609621); and Atrial fibrillation, familial, 3 (607554)	- *Kcnq1* (mouse): head bobbing, intermittent bidirectional circling, and trouble landing on feet/righting themselves; reduced endolymph; shrunken or collapsed vestibular epithelia; hair cell degeneration	- Casimiro et al. (2001) - Winbo and Rydberg (2015)
POU DOMAIN, CLASS 3, TRANSCRIPTION FACTOR 4; POU3F4 (300039)	- Semi-circular canal dehiscence - Reduced vestibular response; mild vestibular hyperactivity (nystagmus) - Associated with deafness, X-linked 2 (304400)	- *Pou4f3* (mouse) impaired balance; absent hair cells in cristae and maculae; loss of vestibular ganglia	- Cremers and Huygen (1984) - Crovetto et al. (2012) - Erkman et al. (1996) - Shine and Watson (1967) - Xiang et al. (1997)

(Continues)

TABLE 1 (Continued)

Human gene name	Possible human behavioral or anatomical phenotypes	Animal model gene name and disease phenotypes	Citations
PROTOCADHERIN 15; PCDH15	- Delayed motor skills associated and vestibular dysfunction - Associated with Usher syndrome, type 1F (602083) and typed 1D/F digenic (601067), and deafness, autosomal recessive 23 (609533)	- *Pcdh15* (mouse): impaired balance; shorter, distorted stereociliary bundles; and degeneration of neuroepithelia	- Ahmed et al. (2003) - Alagramam et al. (2001) - Raphael et al. (2001) - Smith, Lee, et al. (1992) - Zheng et al. (2004)
RIBOSOMAL PROTEIN S26; RPS26 (603701)	- Anomalous vestibule and semi-circular canals - Associated with Diamond-Blackfin anemia 10 (613309)		- McFarren et al. (2007)
SEMAPHORIN 3E; SEMA3E (608166)	- Semi-circular canal agenesis - Associated with CHARGE syndrome (214800)	- *Sema3a* (mouse): defects in the afferent projections of the vestibulocochlear ganglion	- Katayama et al. (2013) - Martin et al. (2001)
SIX HOMEOBOX 1; SIX1 (601205)	- Widened semi-circular canals; enlarged vestibular aqueduct; Mondini dysplasia - Associated with Branchio-otic syndrome 3 (608389) and deafness, autosomal dominant 23 (605192)	- *Six1* (mouse): head-bobbing and absence of righting reflexes; inner ear agenesis; truncated semi-circular canals; reduced number of utricular hair cells; missing hair cells in ampullae	- Ceruti et al. (2002) - Bosman et al. (2009) - Li et al. (2003) - Ruf et al. (2004)
SMALL NUCLEAR RIBONUCLEOPROTEIN POLYPEPTIDES B AND B1; SNRPB (182282)	- Agenesis of the inner ear - Associated with Cerebrocostomandibular syndrome (CCMS) (117650)		- Hennekam and Goldschmeding (1998)
SOLUTE CARRIER FAMILY 26, MEMBER 4; SLC26A4 (605646)	- Enlarged vestibular aqueduct - Vertigo - Associated with Pendred syndrome (274600) and deafness, autosomal recessive 4, with enlarged vestibular aqueduct (600791)	- *Pds* (mouse): head tilting and bobbing, circling, unsteady gait, and abnormal reaching response; endolymphatic dilation; abnormal otolith mineralization	- Dror et al. (2010) - Everett et al. (2001) - Stinckens et al. (2001) - Tsukamoto et al. (2003)
SOLUTE CARRIER FAMILY 12, MEMBER 2; SLC12A2 (600840)	- Delayed motor skills associated with vestibular dysfunction; inability to walk - Associated with deafness, autosomal dominant 78 (619081), Delpire-McNeill syndrome (619083), and Kilquist syndrome (619080)	- *Slc12a2* (mouse): head bobbing, bidirectional circling, and difficulties with locomotion; collapse of vestibular compartments and epithelia	- Delpire et al. (1999) - Dixon et al. (1999) - Flagella et al. (1999) - McNeill et al. (2020) - Mutai et al. (2020)
SORTING NEXIN 10; SNX10 (614780)	- Sclerosis of the semi-circular canals - Associated with autosomal recessive osteopetrosis 8 (OPTB8) (615085)		- Aker et al. (2012)
SRY-BOX 10; SOX10 (602229)	- Semi-circular canal hypoplasia or agenesis - Enlarged central vestibule - Atresia of the vestibulocochlear nerve - Associated with Waardenburg syndrome, type 2E, with or without neurologic involvement (611584); Waardenburg syndrome, type 4C (613266); and PCWH syndrome (609136)		- Elmaleh-Bergès et al. (2013) - Sznajer et al. (2008)
TRANSCRIPTION FACTOR AP2-ALPHA; TFAP2A (107580)	- Enlarged vestibule and vestibular aqueduct - Associated with branchio-oculo-facial syndrome (113620)	- *Ap-2* (mouse): dysmorphogenesis of the inner ear - *tfap2a* (zebrafish): inhibition of neurogenesis and reduced neural maturation	- Kantarci et al. (2015) - Schorle et al. (1996) - Tekin et al. (2009)

TABLE 1 (Continued)

Human gene name	Possible human behavioral or anatomical phenotypes	Animal model gene name and disease phenotypes	Citations
USH1 PROTEIN NETWORK COMPONENT HARMONIN; USH1C (605242)	- Delayed motor skills associated with vestibular dysfunction; difficulty walking - Associated with Usher syndrome, type 1C (276904), and deafness, autosomal recessive 18A (602092)	- Ush1c (mouse): splayed/disorganized stereociliary bundles; reduced response to auditory/vibrational stimuli, hyperactivity, head tossing, and circling behavior - ush1c (zebrafish): reduced number of and disorganized stereociliary bundles; circling swimming behavior; and reduced or abnormal evasive swimming behavior	- Lentz et al. (2007) - Phillips et al. (2011) - Smith, Pelias, et al. (1992)
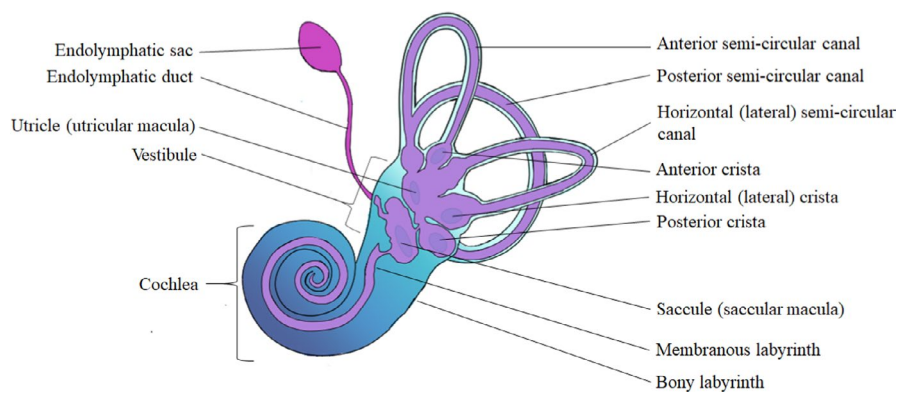
FIGURE 1 Schematic illustration of the adult human inner ear, with an emphasis on the vestibular apparatuses (not to scale)
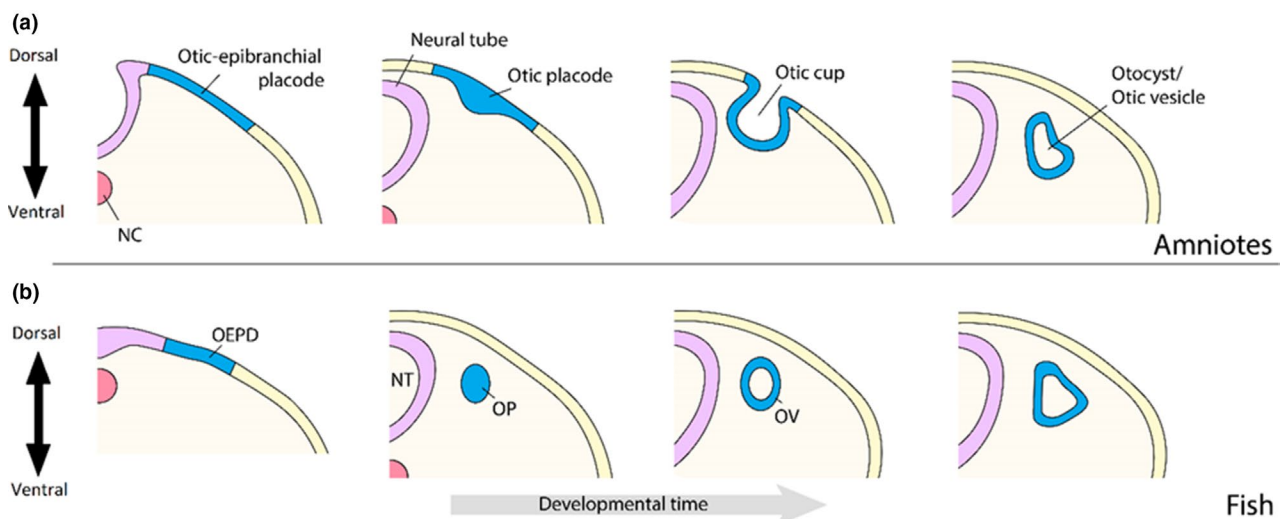
FIGURE 2 Early inner ear development. The inner ear is formed within the pre-placodal region of the ectoderm that is first segregated into the otic/epibranchial precursor domain. A portion of this domain will then give rise to the otic placode, a thickening of the endoderm that invaginates in amniotes (a) or cavitates in fish (b) to form the otocyst/otic vesicle. This vesicle will be subjected to signaling cues that confer various otic identities on the vesicle and shape it into a labyrinth structure that houses both vestibular and auditory apparatuses

ectodermal thickening known as the otic placode. Studies in zebrafish have demonstrated that bone morphogenetic protein (BMP) signaling in the late blastula and early gastrula stages of development establish the competence of the pre-placodal region (PPR) within the ectoderm at the neural plate border where the otic placode originates by inducing the expression of the inner ear marker *foxi1* and other transcription factors (Figure 2; Kwon et al., 2010). It is also known that the PPR does not expand from the head into the embryonic trunk due to canonical WNT signaling, which instead promotes neural crest formation posterior to this placodal region (Litsiou et al., 2005). Subsequently, PPR specification is cooperatively achieved by the dorsal expression of fibroblast growth factors (FGF) and platelet-derived growth factors (PDGF), which act as BMP antagonists. Studies using the aquatic frog *Xenopus* have unveiled another key player for PPR specification, the retinoic acid (RA) signaling pathway, which both restricts the posterior domain of *fgf8* expression in the PPR and induces the expression of the posteriorly restricted PPR genes *tbx1* and *ripply3* (Arima et al., 2005; Janesick et al, 2012; Shiotsugu et al., 2004).

Following PPR induction, this region is segregated into several domains fated for the placodal development of different sensory tissues, such as the otic/epibranchial precursor domain that gives rise to both the otic and epibranchial placodes (Figure 2; Freter et al., 2008; Ohyama et al., 2006; Schlosser & Ahrens, 2004). This entire domain is defined in zebrafish by the ectodermal expression of the Pax transcription factors, *pax2a* and *pax8*, although the subsequent gradient expression of *pax2a* within this region during somitogenesis further defines the individual placodal fates in zebrafish alone; lower levels favor an epibranchial fate, while higher levels direct otic fate as promoted by canonical WNT signaling (McCarroll et al., 2012; Ohyama et al., 2006). However, in *Xenopus* and chick, otic placodal fate relies instead on the expression of *Gbx2* (Steventon et al., 2012). Interestingly, *Pax2/8* double null mutant mice are able to develop an otocyst, although further morphogenesis of the inner ear does not proceed and sensorineural development is severely impaired (Bouchard et al., 2010). It is also important to note that while various cell fates are not specified until later patterning of the ear, studies in zebrafish demonstrate that *foxi1*, which enables the expression of *pax8* at this time, provides otic cells with the competence to embark on a neuronal fate, and *dlx3b/4b*, which subsequently enable *pax2a* activation, then promote sensory fate while restricting neuronal fate (Hans et al., 2013; Hans et al., 2004). Other factors implicated in otic induction that are highly conserved across vertebrates include Fgf3 and Fgf8, with additional FGFs participating in this process in a species-dependent manner (Alvarez et al., 2003; Freter et al., 2008; Ladher et al., 2005; Léger & Brand, 2002; Liu et al., 2003; Mansour et al., 1993; Maroon et al., 2002; Martin & Groves, 2006; Maulding et al., 2014; Padanad et al., 2012; Park & Saint-Jeannet, 2008; Phillips et al., 2001; Wright & Mansour, 2003). While the loss of activity from any one FGF gene does not appear to prevent otic induction, impairing at least two members of this family of signaling molecules will result in a complete loss or deficiency of otic tissues.

Once the otic placode is induced, it invaginates into an otic cup in amniotes (Figure 2a) or cavitates in fish (Figure 2b) to then form the otic vesicle/otocyst (Figure 2; Kaufman, 1992; Maroon et al., 2002; Schlosser & Northcutt, 2000). While the molecular mechanisms behind its cavitation in fish are yet unknown, it is understood that the invagination of this vesicle in amniotes first requires an expansion at the basal aspect of the otic cells, followed by an apical constriction (Sai et al., 2014). Both stages are driven by the activation of myosin-II, although it triggers the depolymerization of actin basally, in response to localized FGF signaling, and actomyosin contraction apically, which is a RhoA-dependent process (Sai & Ladher, 2008; Sai et al., 2014).

5 | AXIAL PATTERNING OF INNER EAR

During and after vesicle formation, otic cells will take on a neural, sensory, or non-sensory cell fate. Cells destined to take on a neural or sensory fate originate in the neural–sensory-competent domain (NSD) that originates within the anterior region of the developing vesicle (Fekete & Wu, 2002b). Cells within this region that express *Neurog1*, a key basic helix–loop–helix (bHLH) transcription factor in neuronal determination, are specified to become neuroblasts, which delaminate from the vesicle ventrally, form a transient amplifying population, and give rise to the afferent neurons of the vestibulocochlear (VIII) cranial ganglion, whereas cells that instead express *Neurod1* are later fated to give rise to hair cells (Ma et al., 1999; Matei et al., 2005; Sapède et al., 2012). The posterior region of the otic vesicle gives rise to predominantly non-sensory tissues, although the posterior crista is eventually situated within this area. When considering how the anteroposterior asymmetry of the ear is determined and differential cell fates are directed along this axis, the RA and FGF signaling pathways have been identified as key factors (Bok et al., 2011; Maier & Whitfield, 2014). Studies in zebrafish and chick show that the expression of *fgf3* in the hindbrain confers an anterior identity within the otic placode, while RA signaling, induced by the expression of *aldh1a3* in the head mesenchyme posteroventral to the placode, promotes the posterior expression of *tbx1*, a negative regulator of neurogenesis within the ear (Figure 3a; Bok et al., 2011; Maier & Whitfield, 2014; Radosevic, 2011; Radosevic et al., 2011; Raft et al., 2004).

Once the otic vesicle is formed, FGF and RA signaling continue to maintain anteroposterior patterning of the ear (Maier & Whitfield, 2014). Both within the anterior aspect of the vesicle and directly ventral to it, the expression of FGFs in zebrafish induces the expression of *aldh1a3* within the posterior region of the NSD to create a localized source of RA signaling (Figure 3a). At this point, FGF and RA create a negative feedback loop that regulates the development of sensory hair cells. More precisely, high FGF levels of signaling in the anterior otic vesicle promote the expression of *otx1b*, which restricts neurogenesis and promotes the development of structural cells, with lower levels allowing for the development of sensory cells. Concurrently, RA signaling both restricts *otx1b* expression posteriorly to permit the
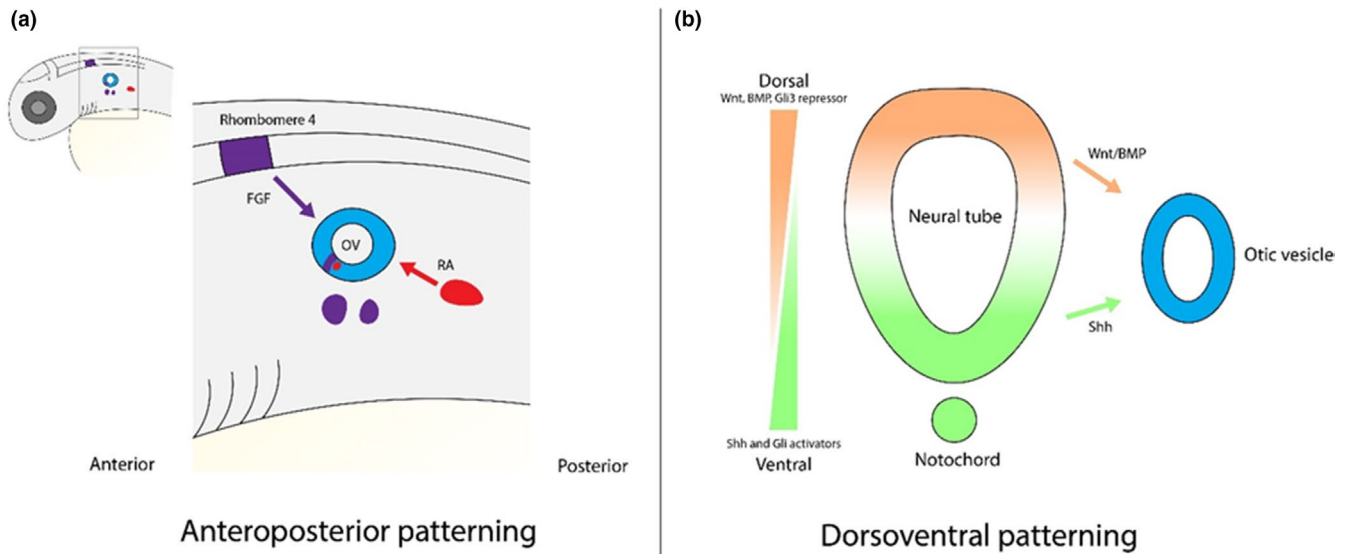
FIGURE 3 Axial patterning regulators of the inner ear. (a) The fibroblast growth factor (FGF) and retinoic acid (RA) signaling pathways are responsible for conferring anteroposterior identities during early inner ear development. Initially, *fgf3* from the hindbrain confers anterior identities within the placode while RA, metabolized by *aldh1a3* in the head mesenchyme posteroventral to the placode, promotes posterior identities. Once the otic vesicle forms, it continues to be subjected to FGF and RA signaling, particularly within the neuro-sensory domain of the vesicle; at this time, FGFs are also expressed ventral to the ear. (b) Dorsoventral patterning of the ear is regulated by the Wnt, Bone morphogenetic protein (BMP), and sonic hedgehog (SHH) signaling pathways. SHH, secreted from the floor plate of the hindbrain and the notochord, is mediated by opposing gradients of the Gli3 repressor within the dorsal epithelium of the otic vesicle and Gli2/3 activators within the ventral domain. Wnt signaling is driven by the secretion of ligands from the dorsal hindbrain, whereas BMP signaling is driven by the secretion of ligands from the dorsal hindbrain and within the otic epithelium. Both Wnt and BMP have been implicated in restricting SHH signaling to the ventromedial region of the ear; ultimately, SHH acts as a ventralizing agent within the ear while WNT and BMP act as dorsalizing agents

development of sensory cells and instead promotes *tbx1* expression to allow for the maturation of otic neuroblasts. However, the FGF and RA signaling pathways are not the only players in anteroposterior patterning and neurogenesis. Sonic hedgehog (SHH) signaling is also believed to be involved in this process, as murine *Shh*^{−/−} mutants display reduced *Neurog1* and *Neurod1* expression, although the underlying mechanism by which this pathway regulates these genes is not yet well understood (Riccomagno et al., 2002).

Patterning of the inner ear is not limited to the anteroposterior axis. Sonic hedgehog (SHH), secreted from the floor plate and notochord, has been identified as a key regulator of dorsoventral identities, as mediated by opposing gradients of the Gli3 repressor within the dorsal domain of the otic epithelium and Gli2/3 activators ventrally (Figure 3b; Bok et al., 2007; Brown & Epstein, 2011). An absence of SHH signaling during inner ear development, as achieved in mouse embryos mutant in *Smo*, an essential transducer of this pathway, results in a wide range of morphological abnormalities later in otic development (Brown & Epstein, 2011). These abnormalities include missing ventral otic derivatives, such as the saccular macula and cochlear duct, and the malformation or absence of dorsal derivatives, such as the utricular macula, endolymphatic duct, and semi-circular canals. Complimentary to these data, ectopic SHH signaling within and around the inner ear results in a complete absence of vestibular structures and a malformed cochlear duct (Riccomagno et al., 2002). Through these studies, it has been determined that SHH is required for specifying otic cells within the

ventral region that would normally express the markers *Otx1*, *Otx2*, and *Pax2*, as *Shh*^{−/−} mutants display reduced or absent expression of these genes. Ectopic SHH signaling has demonstrated that it also regulates cochlear formation through *Brn4* and *Tbx1* expression in the periotic mesenchyme, expanding the former ventrally and the latter dorsally, and through *Pax2* expression in the otic epithelium, which is expanded from the ventromedial region to throughout the vesicle. However, SHH does not act alone in determining dorsoventral identities. The canonical WNT signaling pathway has also been implicated as a key player in specifying dorsal otic fates, with a loss of signaling during otic development resulting in missing vestibular structures, although due to the fact that some cells populating the cochlea are presumed to be exposed to WNT signals early on in otic development, a truncation of the cochlear duct is also observed (Riccomagno et al., 2005). As to how WNT specifics dorsal fates, this is accomplished through the secretion of the canonical WNT ligands *Wnt1* and *Wnt3a* from the dorsal hindbrain (Figure 3b). In *Wnt1*^{−/−}; *Wnt3a*^{−/−} mutants, there is a loss of *Dlx5*, *Dlx6*, and *Gbx2* expression in the dorsal region. Conversely, increased WNT signaling, as achieved through lithium chloride treatment, results in the ventral expansion of these genes in wild-type embryos and rescued expression in *Wnt1*^{−/−}; *Wnt3a*^{−/−} mutants. When taking into consideration any possible cross talk between SHH and WNT at this stage of development, the observed dorsal expansion of the SHH effector *Gli1* in *Wnt1*^{−/−}; *Wnt3a*^{−/−} mutants suggests that WNT acts to restrict SHH signaling to the ventromedial region of the ear. However, the

failed restriction of SHH signaling with lithium chloride treatment in these mutants implies that WNT does not act alone in this process.

More recently, the BMP pathway has been suggested to aid in dorsal specification, as seen with the loss of one or more semicircular canals with reduced signaling in chick embryos, as well as the restriction of SHH in the ear (Ohta & Schoenwolf, 2018; Ohta et al., 2016). BMP ligands expressed in the dorsal hindbrain and otic epithelium promote the expression of the dorsal markers *Hmx3*, through the non-canonical activation of cAMP-dependent Kinase A (PKA), and *Dlx5*, through the canonical Smad-dependent pathway (Figure 3b; Ohta et al., 2016). Because the PKA phosphorylation of Gli results in the processing of Gli repressors, non-canonical BMP signaling can negatively regulate SHH through the reduced expression of the SHH target gene *Otx2* via the overexpression of the BMP ligand *Bmp4*. Additionally, loss of BMP signaling in the otic epithelium results in the expansion of both *Otx2* and *Pax2* expression. Together, this suggests that there is an intricate network of cross talk between these pathways, where SHH acts as a ventralizing agent that favors the development of the auditory structures of the ear while WNT and BMP act as dorsalizing agents that predominantly promote the development of the vestibular structures of the ear.

The final axis of the inner ear is the medial-lateral, although little is known about how it is established. Research in chick embryos shows that some of the genes already discussed, such as *Pax2* and *Gbx2*, have distinct dorsomedial expression patterns in the ear (Brigande et al., 2000; Choo et al., 2006). Interestingly, the *Pax2* expression domain is thought to mark one side of the medial-lateral boundary of the inner ear, on the other side of which we see the ventral-lateral expression of *SOHo* (Brigande et al., 2000). In fact, the dorsal boundary between the expression pattern of these two genes marks the location of the endolymphatic duct's outgrowth, and *Pax2⁻/⁻* mutants display malformed endolymphatic ducts that are fused with the common crux, the branching point between the anterior and posterior semi-circular canals, and which are often not properly restricted from the saccular compartment (Bouchard et al., 2010; Brigande et al., 2000; Burton et al., 2004). *Gbx2⁻/⁻* mutants also display abnormalities of this channel, most commonly in the form of an absent endolymphatic duct (Choo et al., 2006). Less is known about how lateral structures of the ear are specified, but fate-mapping studies in chick through the injection of fluorescent carbocyanine dyes into the cells of the rim of the otic cup suggest that the establishment of the medial-lateral axis depends on the establishment of the other axes first (Brigande et al., 2000). This research suggests that while the otic vesicle/otocyst is forming, the lateral region of the otic vesicle that gives rise to the canals first originates in the dorsal region.

6 | VESTIBULAR NEURONAL AND SENSORIAL DEVELOPMENT

While most genes involved in the specification of different cell fates appear once patterning of the otic vesicle is complete,

neuronal specification occurs as early as the formation of the otic/epibranchial precursor domain, with the early expression of *foxi1* imparting the competence for neuronal cell fate and *dlx3b* and *dlx4b* subsequently promoting sensory fate (Hans et al., 2004, 2013). In mouse embryos, once patterning of the otic vesicle is complete and the NSD is defined by FGF and RA signaling, it is within this region that we see the expression of essential neuronal and sensory markers. As previously described, *Neurog1* and *Neurod1* mark cells within the NSD are fated to become neuroblasts and hair cells, respectively, although mutations in either result in defects of both otic neurons and hair cells (Jahan, Pan, et al., 2010; Ma et al., 1999; Matei et al., 2005; Sapède et al., 2012). In particular, a loss of *Neurog1* in mice results in an earlier hair cell cycle exit, resulting in the failed formation of sensory neurons and a truncated clonal expansion of in hair cell precursors, leading to the development of fewer hair cells overall (Matei et al., 2005). Loss of *Neurod1* results in the formation of hair cells within the sensory ganglia of the inner ear, neuronal death, and the failed segregation of vestibular and cochlear afferents, leading to a projection of a single, mixed nerved to the cochlea (Jahan, Kersigo, et al., 2010; Jahan, Pan, et al., 2010; Macova et al., 2019). Other essential neuronal and sensory markers include *Eya1*, *Six1*, and *Sox2* (Kiernan et al., 2005; Nichols et al., 2008; Xu et al., 1999; Zheng et al., 2003; Zou et al., 2008). Both *EYA1* and *SIX1* have been implicated in Branchio-oto-renal syndrome, which is usually characterized by hearing impairments and kidney and urinary tract malformations but can also include vestibular abnormalities, such as a truncated posterior semi-circular canal or an enlarged endolymphatic sac and duct (Abdelhak et al., 1997; Ceruti et al., 2002; Melnick et al., 1976; Ruf et al., 2004). Studies in mice models have shown that loss of either otic *Eya1* or *Six1* results in the decreased expression of the sensorineural markers *Neuorg1* and *Neurod1* and that their co-overexpression is capable of inducing neurogenesis in the ear (Ahmed, Xu, et al., 2012; Li, Zhang, et al., 2020). *Sox2* has also been determined to be required for the expression of both *Neurog1* and *NeuroD1*, engaging in an inhibitory feedback loop with these genes, and was initially assumed to control the number of neuroblasts that give rise to the afferent neurons of the VIII cranial ganglion (Evsen et al., 2013; Steevens et al., 2017). However, more recent work in mice utilizing the conditional deletion of *Sox2* in otic tissues demonstrates that it does not appear to affect the initial delamination of neuroblasts, although the sensory epithelia of the inner ear ultimately fails to develop and early neurons perish (Dvorakova et al., 2020). It is possible that *Sox2* functions in the developing ear by interacting with either *Eya1* or *Six1*, as they have already been shown to co-operatively promote the expression of other otic markers in cochlear cell cultures (Ahmed, Wong, et al., 2012). This includes *Atoh1*, an essential basic helix–loop–helix (bHLH) transcription factor associated with hair cell differentiation, which is normally suppressed by *Neurod1* to prevent the premature or ectopic differentiation of hair cells (Chen et al., 2002; Jahan, Pan, et al., 2010; Kopecky et al., 2013; Pan et al., 2011). It is important to also note that Notch signaling plays an important

role within the NSD for determining hair cell and to support cell fates (Pan et al., 2013; Petrovic et al., 2014). Initially, as directed through the Notch ligand Jag1, Notch-mediated lateral induction induces *Sox2* expression to specify the prosensory domain and prevent early hair cell differentiation. Once *Atoh1* is expressed, it in turn induces *Dl1*, another Notch ligand. This creates a competition between Di1 and Jag1 for Notch, where the former favors the development of support cells and the latter the development of hair cells, creating a latticed pattern of the two cell types within this sensory domain.

Knowing that the NSD gives rise to the various sensory epithelia found in the mature inner ear, it is important to recognize that medial-lateral patterning plays a role in how the neuroblasts in the pre-segregated domain are determined to form ganglia of either the vestibular or auditory branches of the VIII cranial nerve. It has been discovered that otic cells that express *Fgf3* within the anterior-lateral region of this domain delaminate first and generate vestibular neurons (Bell et al., 2008; Koo et al., 2009). Meanwhile, cells that express *Gata3* and *Lmx1a* within the medial region delaminate later and generate auditory neurons (Karis et al., 2001; Koo et al., 2009; Koundakjian et al., 2007; Lawoko-Kerali et al., 2004). However, another possible player in the process is *Myo7A*, which encodes the unconventional myosin VIIA, a critical motor protein that in chick embryos is expressed in differentiating vestibular neurons before it is subsequently restricted to the vestibular afferents of the cristae (Nguyen et al., 2015). It is possible that *Myo7A*, therefore, acts in the innervation of these afferents or axonal migration and pathfinding, although most research into this gene has focused on the role it later plays in organizing the stereocilia bundles of vestibular and auditory hair cells and, therefore, its connection to Usher Syndrome type 1 in human patients (Ernest et al., 2000; Gibson et al., 1995; Nguyen et al., 2015; Nichols et al., 2008; Weil et al., 1996).

Further studies have demonstrated that NSD separation into the individual sensory epithelia throughout the ear requires restriction of *Lmx1a* expression to the non-sensory in mice, with studies in *Lmx1a/Lmx1b*$^{-/-}$ mice demonstrating a severe disruption in auditory development that results in a complete loss of the organ of Corti, the sensory epithelium of the cochlea (Chizhikov et al., 2021; Nichols et al., 2008). As for the separation between more distinct regions of the initial sensory domain, *Fgf10* appears to play an important role in the separation of the cristae, as mutants lack a posterior crista, only producing reduced and malformed anterior and lateral cristae (Pauley et al., 2003). Another player is *Foxg1*, whose null mutants often have fused anterior and lateral cristae and demonstrate severe abnormalities of both vestibular and auditory innervation (Hwang et al., 2009; Pauley et al., 2006). *Otx1* also seems to play a role in cristae separation, as null mutants in mice and zebrafish lack a lateral crista, as well as a lateral canal (Fritzsch et al., 2001; Hammond & Whitfield, 2006; Morsli et al., 1999). However, these mutants also possess a fused utricle and saccule, which additionally implicates *Otx1* in the proper segregation of the two maculae.

7 | SEMI-CIRCULAR CANAL DEVELOPMENT

The cells that give rise to the semi-circular canals, which are responsible for detecting angular accelerations of the head, originate in a lateral region of the vesicle adjacent to where the presumptive cristae will form (Brigande et al., 2000; Chang, Brigande, et al., 2004). In fact, the cristae are likely responsible for inducing the formation of the canal ducts through the BMP and FGF signaling pathways, as *Bmp4* in mice, chick, and zebrafish is essential for regulating *Bmp2* and *Dlx5*, two key players in canal formation, and *Fgf10*$^{-/-}$ mutants in chick lack all three canals (Chang, Brigande, et al., 2004; Chang et al., 2008; Hammond et al., 2009; Omata et al., 2007; Pauley et al., 2003; Wright & Mansour, 2003). *Fgf3* has also been identified as another FGF involved in canal morphogenesis, as knockouts in mice occasionally lack a posterior canal (Mansour et al., 1993; Pauley et al., 2003).

Although there are morphological variations in how the canals are formed across vertebrates, the end result is three semi-circular canals situated at right angles relative to one another. In zebrafish and *Xenopus*, we see an outgrowth of projections that bulge into the vesicle and fuse at a central point to form the pillars around which the three canals are formed (Figure 4a; Paterson, 1949; Waterman & Bell, 1984). In both of these species, glycosaminoglycan hyaluronan production is essential for these outgrowths, although other factors discovered in zebrafish have also been implicated in the formation of these projections, such as *atropin2*, *atp1a1a.2*, *atp2b1a*, *cdh2*, *grhl2*, *fgf8*, *ncs1a*, and *otx1* (Asai et al., 2006; Babb-Clendenon et al., 2006; Blasiole et al., 2006; Blasiole et al., 2005; Busch-Nentwich et al., 2004; Cruz et al., 2009; Haddon & Lewis, 1991; Hammond & Whitfield, 2006; Han et al., 2011; Neuhauss et al., 1996). More recently, *gpr126*, an adhesion G protein-coupled receptor gene expressed in the projections, has been identified as an important regulator of genes that encode extracellular matrix core proteins or are involved with modifying the extracellular matrix, such as *chsy1*, *has3*, *hapln1a*, *hapln3*, *ugdh*, and *vcana* (Geng et al., 2013). Changes to the extracellular matrix can understandably impact the proliferation or migration of cells within a given tissue, directly affecting tissue morphology, although they could contribute to signal transduction processes. Future research could, therefore, focus on determining which signaling pathways might direct or be directed by such changes to the extracellular matrix during semi-circular canal morphogenesis.

In amniotes, morphogenesis of the semi-circular canals begins with the formation of two out-pouches, one vertical, situated dorsally, and one horizontal, which forms laterally around the middle of the otocyst (Bissonnette & Fekete, 1996; Fekete, 1999; Kopecky et al., 2012). The canonical WNT signaling pathway is believed to play an essential role leading up to this stage in promoting cell proliferation and repressing apoptosis, likely ensuring that a sufficient number of cells are available for the formation of these out-pouches (Noda et al., 2012). With the ventral out-pouch, opposing epithelial extend toward one another to form a fusion plate, which fuses
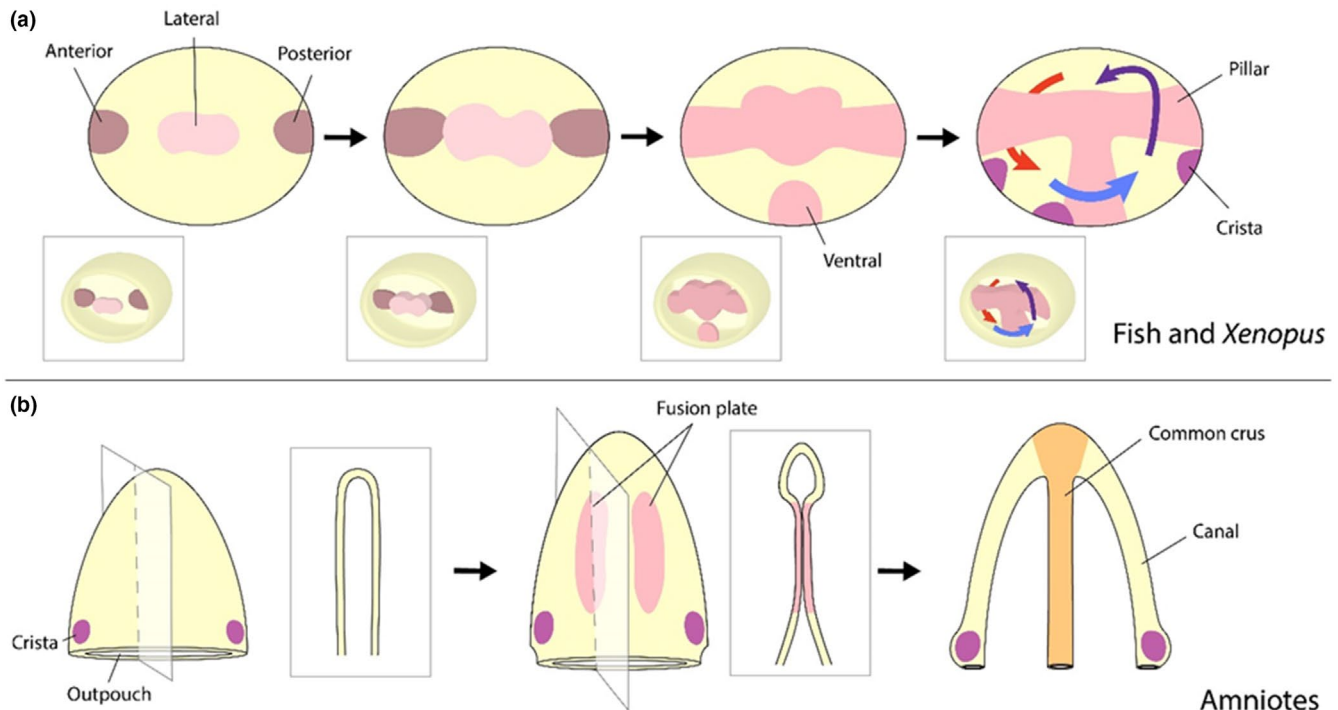
FIGURE 4 Semi-circular canal morphogenesis. The cells that give rise to the canals originate in a lateral region adjacent to the presumptive cristae. However, there are variations between how these canals form in anamniotes, such as zebrafish and *Xenopus*, and amniotes, such as mouse and chick. (a) Canal formation in zebrafish and *Xenopus* begins with an outgrowth of projections that bulge inward into the otic vesicle. When these projections fuse, they become the pillars around which the canals are formed. (b) In amniotes, the canals are sculpted from two out-pouches, one ventral (shown above) and one horizontal. Fusion plates are formed by opposing epithelium that extend toward one another. Following fusion, these plates are resorbed through apoptosis. In the above example, this process forms the anterior and posterior canals, with the common crux situated between them

and then is resorbed through apoptosis to create two canals along the rim, the anterior and posterior, which are connected centrally at the common crus (Figure 4b). The horizontal out-pouch undergoes a similar process of fusion and resorption to give rise to the lateral canal. Despite the division between horizontal canal formation and that of the anterior and posterior canals in amniotes, *Lmo4* in mouse embryos appears to be responsible for the outgrowth of both pouches by regulating cell proliferation and maintaining the expression of many players in canal morphogenesis, including *Bmp4*, *Fgf10*, *Gata3*, and *Dlx5* (Deng et al., 2010). Here also the canonical WNT signaling pathway has been identified as an important factor behind semi-circular morphogenesis for its role in inducing the expression of *Netrin1*, which induces fusion and resorption of the fusion plate by promoting periotic mesenchymal proliferation to push the opposing epithelial together and the detachment of the fusion plate from the basement membrane (Noda et al., 2012; Salminen et al., 2000).

8 | PRODUCTION AND MAINTENANCE OF ENDOLYMPH

During its development, the otic vesicle is filled with endolymph, a fluid that is essential for sensory hair cell function and proper otolith or otoconia composition. With respect to hair cell function,

the endolymph has a high concentration of potassium, which flows through the transducer channels of these cells with the deflection of their stereocilia and thereby triggers their depolarization (Battey, 2000; Bosher & Warren, 1968). Upon depolarization, the hair cells release neurotransmitters at their synapses with the afferent neurons of the vestibulocochlear nerve, the first step in triggering the perception of motion or sound. Some genes in human patients with auditory or vestibular impairments, such as those suffering from Jervell and Lang-Nielsen syndrome and Delpire-McNeill syndrome, have been implicated in controlling the composition or homeostasis of endolymph (Kulkarni et al., 2012; McNeill et al., 2020). Mutations in animal models often resulting in reduced endolymphatic volume and potential, commonly leading to a shrinkage or collapse of the membranous labyrinth and hair cell death (Blasiole et al., 2006; Casimiro et al., 2001; Cruz et al., 2009; Delpire et al., 1999; Dixon et al., 1999; Flagella et al., 1999). These genes include *Slc12a2*, which encodes the basal Na–K–1Cl co-transporter that pumps potassium from the hair cells into the homologous epithelial "dark" cells surrounding the vestibular sensory epithelia, and both *Kcnq1* and *Kcne1*, the subunits of KvLQT1, a channel on the apical side of dark cells responsible for returning potassium to the endolymph (Blasiole et al., 2006; Casimiro et al., 2001; Cruz et al., 2009; Delpire et al., 1999; Dixon et al., 1999; Flagella et al., 1999).

Dark cells, like the marginal cells of the cochlea, are also responsible for producing endolymph during otic development,

although there appears to be some variation in the composition and function of endolymph-producing cells surrounding the various sensory epithelia between species (Dohlman, 1965; Harada et al., 1989; Hommerich, 1990; Kimura et al., 1964; Villegas et al., 2001; Wangemann, 1995). However, one gene that has been found to be essential for the development and function of both dark and marginal cells is the estrogen-related receptor beta, _Nr3b2_ (Chen & Nathans, 2007; Wangemann, 1995). In _Nr3b2$^{-/-}$_ mouse mutants, these cells lack multiple ion channels and transporters and possess the characteristic narrowing of the membranous labyrinth of the semi-circular canals normally found with improper endolymph composition or homeostasis (Chen & Nathans, 2007).

9 | OTOCONIA (OTOLITH) FORMATION AND TETHERING

To enable the detection of gravity and linear accelerations, the maculae rely on a collection of calcium carbonite crystallites embedded in a gelatinous matrix of proteoglycans and proteins that overlay the hair cells (Ross & Pote, 1984). These bio-mineral structures are called otoliths in fish and otoconia in most other vertebrates, and while there are structural differences between the two, there is a significant overlap in the genes responsible for otoconia and otolith formation. Some of these shared genes are involved in endolymph composition, which has a direct effect on the composition of these bio-mineral structures, impacting their size and shape. These include the anion transporter Pendred, which is encoded by _Slc26a4_, and those encoding the enzyme carbonic anhydrase, which are together responsible for maintaining an alkaline endolymphatic pH, primarily through the production or transport of HCO_3^- (Everett et al., 2001; Kido et al., 1991; Kim & Wangemann, 2010, 2011; Nakaya et al., 2007; Tsujikawa et al., 1993). Human patients with mutations in _SLC26A4_ suffer from the characteristic enlarged vestibular aqueduct associated with Pendred syndrome, which typically results in progressive hearing loss and episodic vertigo (Stinckens et al., 2001). Other genes encode proteins that are responsible for the mobilization of calcium, such as _Otop1_, which also participates in protein secretion, and _Atp2b2_, a calmodulin-sensitive plasma membrane calcium-ATPase that increases calcium concentration around the maculae (Blasiole et al., 2006; Hughes et al., 2007; Kim et al., 2010, 2011; Kozel et al., 1998). The loss of either of these genes in animal models results in the complete absence of otoliths or otoconia (Blasiole et al., 2006; Hughes et al., 2007; Kim & Wangemann, 2010, 2011; Kozel et al., 1998).

Other key players in otoconia or otolith formation are the proteins that make up the otolithic membrane, which are collectively referred to as otoconins, although there is some variation in the role each gene plays between species. These encode, for example: Oc90, which recruits calcium and other otoconial matrix components to facilitate crystal nucleation and growth; Otolin-1, a collagen that interacts with Oc90 to act as a scaffold in the same processes; and keratan sulfate proteoglycans, which interact with both Oc90 and

Otolin-1, as well as other proteoglycans and collagens, to attract calcium (Deans et al., 2010; Killick & Richardson, 1997; Lu et al., 2010; Petko et al., 2008; Xu et al., 2010; Yang, Zhao, et al., 2011; Zhao et al., 2007). Null mutants in mice and zebrafish in these genes often result in lost or greatly reduced otoconia or otoliths (Deans et al., 2010; Killick & Richardson, 1997; Lu et al., 2010; Murayama et al., 2005; Petko et al., 2008; Xu et al., 2010; Yang, Zhao, et al., 2011; Zhao et al., 2007).

Another important step in otoconia or otolith formation is the initial tethering of these bio-crystals to their underlying sensory epithelia, a task shared by various glycoproteins. Two such glycoproteins are otogelin, which is collagenous, and otogelin-like, which is non-collagenous, and which together are necessary for both the anchoring of the otolithic membrane to the maculae and the anchoring of the cupula to the crista of the semi-circular canals (Bonnet et al., 2013; Cohen-Salmon et al., 1997; Simmler, Cohen-Salmon, et al., 2000; Simmler, Zwaenepoel, et al., 2000; Yariz et al., 2012). Other important factors for proper tethering include _α-Tectorin_ and _β-Tectorin_, which each encode non-collagenous glycoproteins that are expressed in the macular sensory epithelia (Legan et al, 1997; Rau et al., 1999). In mouse and zebrafish studies, null mutants of _α-Tectorin_ have scattered otoconia and reduced otolithic membrane, whereas _β-Tectorin_ have fewer or fused otoliths (Legan et al., 2000; Yang, Cheng, et al., 2011). Interestingly, analysis of the acellular membrane overlying the auditory sensory epithelium of the inner ear, the tectorial membrane, demonstrates that it also contains _α-Tectorin_ and _β-Tectorin_ (Goodyear & Richardson, 2002). Studies in mice demonstrate that a loss of _α-Tectorin_ results in a decoupling of the tectorial membrane from the underlying sensory epithelium, leading to impaired sound amplification and frequency-dependent hearing loss (Legan et al., 2000). Oddly enough, _Tectb$^{-/-}$_ mice lead to an increased sharpness in cochlear frequency tuning, although there is a loss of sensitivity for frequencies below 20Hz, presumably from an enlargement of the tectorial membrane at the apical end of the cochlea (Russell et al., 2007).

10 | OVERVIEW OF THE EVOLUTION OF THE VERTEBRATE INNER EAR

To understand the development of an organ, it is important to consider the changes that it has undergone over the course of its evolution. While we are still piecing together the evolutionary steps behind the development of the inner ear, the aim of this section is to touch upon some of the theories behind the origin of this sensory organ and how variations in its anatomy developed to suit the needs of different species.

11 | EVOLUTION OF THE SPECIALIZED ENDORGANS OF THE INNER EAR

Although the "inner ear" is considered a solitary organ, it contains multiple apparatuses specialized in the detection of different stimuli.

In general, the cochlea detects sound, the semi-circular canals detect angular accelerations, and the maculae detect gravity and linear accelerations, raising the question of which apparatus came first. Up until the late 1800s, the inner ear was believed to serve a purely auditory function, with the semi-circular canals and maculae supplementing the detection of sound by acting as a type of intensity coding system (Haeckel, 1877). However, lesions of the inner ear and later physiological observations revealed that the canals instead served a crucial purpose in detecting rotations of the head while the otoliths were involved with the perception of gravity (Breuer, 1873; Mach, 1873). Subsequently, it was discovered that while aquatic vertebrates possessed a structured labyrinth similar to that of land vertebrates, the anatomy of their inner ear did not immediately suggest the presence of a clearly analogous auditory apparatus to our own, which was the first indication that a specialized auditory system was a more recent acquisition (Retzius, 1881). While we now know that aquatic vertebrates are capable of detecting sound with their otolith organs, the evolution of a more intricate auditory system better capable of detecting airborne sounds would have been necessary for terrestrial tetrapods to avoid predators or track prey on land (Christensen, Christensen-Dalsgaard, et al., 2015; Christensen, Lauridsen, et al., 2015; Christensen-Dalsgaard et al., 2011; Clack, 2015; Lu & Xu, 2002; Lu et al., 2003, 2010).

Given its close proximity to the macular recess, the auditory sensory epithelium of the inner ear, known as the organ of Corti in mammals and the basilar papilla in amphibians, reptiles, and birds, is believed to have evolved from the otolith organs (Fritzsch & Wake, 1988). The evidence behind this theory is made apparent when considering the morphological similarities between the hair cells of each apparatus. Analyses of the sensory epithelia of the vestibular system reveal that two kinds of hair cells can be found in both the maculae and the ampullae, which are simply described as "type I" and "type II." Type II hair cells occur in all vertebrates and are believed to be evolutionarily older, while type I hair cells are only found in birds, mammals, and reptiles (Wersall, 1956; Wersäll & Bagger-Sjöbäck, 1974). Type I hair cells possess a flask-shaped body, calyceal ("cup-like") afferent nerve endings that encompass the basolateral surface of one or more hair cells, and efferent synapses that contact the calyx rather than the hair cell itself; type II hair cells possess more cylindrical bodies, connect with multiple afferent bouton synapses, and contact their efferent synapses directly (Deans, 2013; Eatock et al., 1998; McInturff et al., 2018; Wersäll & Bagger-Sjöbäck, 1974). Type II hair cells also have fewer stereocilia per bundle (Peterson et al., 1996; Wersäll & Bagger-Sjöbäck, 1974). The resting potential of type I hair cells is also incredibly negative, and their primary current is a large and non-activating K+ delayed inward rectifier, which allows these cells to produce faster voltage responses (Correia & Lang, 1990; Eatock & Songer, 2011; Meredith & Rennie, 2016; Rennie & Correia, 1994; Rusch & Eatock, 1996). The calyceal afferent synapses of type I cells also make these hair cells capable of much a faster, non-quantal ("non-vesicle") form of transmissions that involve K+ accumulation in the synaptic cleft and ion channels in the synaptic membranes that face this cleft (Eatock, 2018). By comparison,

type II hair cells have a more depolarized membrane potential and smaller activation range, although this is believed to increase their sensitivity (Correia et al., 1996). Both of these hair cells also possess ribbon synapses, a type of synapse that is unique to sensory tissues, differentiated from chemical synapses by a specialized organelle referred to as the "ribbon" that holds synaptic vesicles close to the active zone of the cell (Goldberg et al., 1990; Lysakowski & Goldberg, 1997; Matthews & Fuchs, 2010; Odermatt & Lagnado, 2009). This ribbon is thought to transport these vesicles to the site of fusion in an inconcurrent manner, allowing for the more sustained release of neurotransmitters (Odermatt & Lagnado, 2009).

The sensory epithelium of the auditory system is likewise composed of two types of hair cells: inner hair cells, which are the primary auditory receivers, and outer hair cells, which both mechanically amplify low-intensity sound and are involved with sharpening frequency tuning curves (Aitkin, 1995; Ashmore, 1987, 2008; Dallos, 1996; Kössl & Vater, 1995; Ladhams & Pickles, 1996; Wever et al., 1971). The morphology of inner hair cells is that of a flask-shaped body with a thick stereociliary bundle, like that of type I hair cells, while outer hair cells are more cylindrical and possess fewer stereocilia, like that of type II hair cells (Forge et al., 1991; Schwander et al., 2010; Slepecky, 1996). Both hair cells also possess ribbon synapses, although neither of these cell types possess the calyceal afferent synapses seen in the vestibular system (Francis et al., 2004; Khimich et al., 2005; Liberman, 1980; Liberman et al., 1990). As vertebrates transitioned from an aquatic to a terrestrial environment, the elongation of the limbs and neck allowed for a wider range and more abrupt motions of the head and body (Biewener, 1983; Ericsson et al., 2013; Kear et al., 2008; Young, 2013). However, this transition brought with it an increased risk of injury from falling. Although we still have much to learn about how calyceal synapses came to be, one of the driving forces behind their evolution solely within the vestibular system could be to provide faster reflexes in a terrestrial environment (Bierbower & Cooper, 2013; Korn & Faber, 2005).

Looking to the acellular membranes of the sensory epithelia of the inner ear, we can also glean something about the evolution of the various apparatuses of the inner ear. The tectorial membrane of the cochlea contains both α-Tectorin and β-Tectorin, which are utilized in the striolar region of the macula for otolith or otoconial tethering (Goodyear & Richardson, 2002). The otolithic membrane and tectorial membrane of the basilar papilla are also attached to their respective sensory epithelia by fibrous veils. These shared traits further support the notion that the auditory sensory epithelium evolved from the macular striola. However, an analysis of these acellular membranes also raises an interesting point about whether the macula or the ampulla predate one another. For example, one of the most prominent theories of otic evolution suggests that the inner ear originated from the lateral line system due to the close proximity of their placodes during development and the similar structure of the lateral line neuromasts to the ampullae that being of a group of hair cells embedded in a cupular membrane (Fritzsch et al., 1998; Popper et al., 1992; Wever, 1974). The cupular membrane of the inner ear is also less complex than that of the otolithic membrane,

lacking either of the tectorins and being composed of randomly oriented filaments versus the densely and more aligned filaments of the otolithic membrane (Goodyear & Richardson, 2002). However, gravioreceptive sensors appear much earlier in history. Cnidarians, such as jellyfish, possess statocysts, small sensory vesicles that contain heavy crystal ("statolith")-bearing cells that perceive both the direction of gravity and water currents (Park et al., 2012; Schafer, 1878). The semi-circular canals do not appear until much later with the arrival of the chordates, whose increasingly dynamic lifestyles required a more complicated gaze and posture stabilizing system (Higuchi et al., 2019; Mazan et al., 2000; Park et al., 2012). This, therefore, argues that the inner ear likely originated from a similar gravioreceptive sensor, from which additional, more specialized endorgans appeared to enable the refined detection of sound and motion (Duncan & Fritzsch, 2012; Wever, 1974).

Analysis of the sensory epithelia of the inner ear also provides us with clues about the development of its non-sensory tissues (Fritzsch & Straka, 2014). For example, inductive signals from the sensory epithelium, such as Bmp4 and Fgf10, drive semi-circular canal morphogenesis, demonstrating a reliance of non-sensory development on inductive signals from sensory tissues and highlighting the fact that the evolution of the cristae predates that of canal formation (Chang, Brigande, et al., 2004; Chang et al., 2008; Pauley et al., 2003). In fact, no mutants bearing inner ear defects have been discovered in which the semi-circular canals form normally when sensory development is perturbed (Anagnostopoulos, 2002; Chang, Cole, et al., 2004; Whitfield et al., 1996). Furthermore, it has even been demonstrated that the induction of hair cells is enough to generate vesicles *in vitro*, mirroring one of the initial steps of inner ear development (Koehler et al., 2013). Even within the sensory epithelium itself, the specification and organization of non-sensory structures relies on the proper sensory development of their neighboring cells. For example, null mutant mice of *Neurog1* demonstrate a substantial reduction in the number of hair cells in all sensory epithelia of the inner ear; meanwhile non-sensory structures such as the cruciate eminence, a division that appears within the cristae of some species, are converted into hair cells (Harada, 1972, 1974; Ma et al., 2000; Matei et al., 2005). While there is still a great deal to learn concerning how otic sensory and non-sensory tissues communicate with one another, the secret to how a simple vestibular organ developed into a more intricate labyrinth likely resides within its mechanosensory hair cells (Duncan & Fritzsch, 2012).

12 | EVOLUTION OF THE SEMI-CIRCULAR CANALS AND COMPLEX MOTOR BEHAVIORS

Although the labyrinthine nature of the inner ear is often considered a "vertebrate" invention, we now know the intricate structure of this sensory organ belongs to the chordates, as even early cyclostomata were found to have distinct semi-circular canals (Wever, 1974). Therefore, by looking to the Agnatha, the sister group of jawed

vertebrates, we can perhaps further understand how the inner ear more recently changed over the course of evolution to produce the characteristic three-canal labyrinth of the gnathostomes and how these changes then enabled a more complex repertoire of motor behaviors.

Instead of the three orthogonally oriented canals found in gnathostomes, both ostracoderms, which are the extinct armored jawless fish of the Paleozoic, and lampreys possess only two canals, each terminating in an ampulla (Higuchi et al., 2019; Mazan et al., 2000). These canals are homologous to the anterior and posterior canals found in jawed vertebrates, suggesting that the lateral canal is a more recent acquisition. Hagfish, the only other modern representative of agnathans besides lampreys, instead possess one ring-like semi-circular canal predicted to have formed around a solitary epithelial projection, much in the same way the canal pillars form in zebrafish or *Xenopus* (Chagnaud et al., 2017; Higuchi et al., 2019; Paterson, 1949; Waterman & Bell, 1984). While this initially suggests that the one-canal morphology is an ancestral trait, hagfish possess two ampullae located at opposite ends of their ring-like canal. Following the "one ampulla per canal" theory, this instead proposes a two-canal situation in our common ancestor, suggesting that a secondary fusion of these canals occurred in hagfish over the course of evolution (Goodrich, 1909). Even though we are uncertain about the true ancestral state, a recent comparative analysis between extant cyclostomes has reinforced what we know about the highly conserved molecular mechanisms behind inner ear development (Higuchi et al., 2019). For example, in lampreys, it has been discovered that *Tbx1/10A* is expressed in the posterior domain of the otic vesicle, where it plays a role in specifying the anteroposterior axis of the inner ear by suppressing genes involved in the neurogenesis and de-epithelialization of vestibular ganglionic cells, much like its homolog in jawed vertebrates, *TBX1* (Higuchi et al., 2019; Radosevic, 2011; Radosevic et al., 2011; Raft et al., 2004). Additionally, dorsoventral specification of the lamprey ear is reliant on Hedgehog signaling, much as it is in gnathostomes, with the expression of *patched A* originating in the ventral region of the vesicle (Higuchi et al., 2019). However, why cyclostomes failed to develop a lateral canal has yet to be determined. It was once assumed that the OTX family of transcription factors were responsible for lateral canal development, as mice mutant in *Otx1* are missing this third canal, but the expression of *OtxA* within the ventral domain of cyclostomes suggests that these transcription factors alone cannot account for the evolution of this canal (Acampora et al., 1998; Higuchi et al., 2019; Morsli et al., 1999).

Beyond their use in comparative molecular analyses, studies of the inner ear of the Agnatha also provide valuable insight into the evolution of more complex motor behaviors. For example, one question that the Agnatha allows us to address is whether three orthogonally oriented semi-circular canals are required for the detection of rotational accelerations on all three axes. To reiterate, lampreys possess only two canals, lacking the lateral canal found in gnathostomes responsible for detecting horizontal accelerations of the head, which would initially imply that they are unable to sense horizontal

movement (Maklad et al., 2014). However, current analyses of their locomotion, a rhythmic undulation of the head and body on the horizontal plane, suggest that they would, in fact, require the perception of horizontal accelerations to move efficiently. Additionally, while the innervation of the lamprey eye is unique, they possess caudal and rostral recti muscles, implying that they should be capable of the vestibule–ocular reflex (i.e., stabilization of the eye) during horizontal movements of the head (Cohen, 1974; Maklad et al., 2014). In fact, a more in-depth analysis of the electrophysiological response of their vestibulocochlear nerve shows that stimuli in all planes, including the horizontal, are capable of eliciting a response, even though the response from each of its two ampullae is somewhat modified from what is observed in higher vertebrates (Maklad et al., 2014). To summarize, this supports the idea that lampreys are able to perceive and react to stimuli on the horizontal plane, despite lacking a lateral canal, although the evolution of a lateral canal likely aided in the refined detection of stimuli on this plane.

Given that three unique canals are not required for sensing motion in a three-dimensional space, this raises the question of how other changes in otic anatomy translate to differences in behavior. Studies have shown that the "radius of curvature" of a canal, and therefore its overall size, plays a significant role in the agility of a species (Spoor et al., 2007). For example, it is found that primates and other mammals, such as galagos and tarsiers, possess a large canal radius relative to body mass, enabling them to move faster and with less circumspection than other mammals, such as extant sloths, which are well known for their slow locomotion (Billet et al., 2013; Spoor et al., 2007). This correlation extends beyond mammals, such that when comparing fish with land-based vertebrates of the same size, a much greater curvature to body mass ratio is found, corresponding to a greater sensitivity to angular accelerations (Jones & Spells, 1963). However, these species all possess an orthogonal three-canal system. When considering a species such as the hagfish, which have only a single vertical canal, it is interesting to see what other anatomical compensations need to be made to allow for efficient movement (Higuchi et al., 2019; McVean, 2009). Hagfish also make a fascinating research subject for this topic of study because they do not possess functional eyes, or do they have a conventional "neck," which means the degree to which their solitary canal needs to measure angular accelerations is not reliant on their ability to stabilize their gaze when they are in motion (McVean, 2009). However, electrophysiological analyses indicate that hagfish can still detect rotational accelerations and on more than one axis, although with less sensitivity, particularly on the horizontal plane. One reason their single canal detects accelerations on multiple axes could be that, unlike other fish, the internal radius of their canal is much larger than its radius of curvature. However, this anatomical difference might actually exist to compensate for the lack of cupula overlying the ring-like crista of the hagfish ear, although it was originally thought that the cupula serves to stabilize and protect the hair cell bundles within the ampullae (Dohlman, 1980). During an acceleration, this cupula is displaced, which deflects the embedded hair cells within and triggers a response. It is, therefore, hypothesized

that a canal's radius of curvature must be large enough to reduce the time required for the cupula to return to its resting position and, therefore, allow the embedded hair cells to detect the next acceleration. In hagfish, this requirement for a large radius of curvature might simply not exist due to the lack of cupula (McVean, 1991). Altogether, while these studies have given us an idea of how inner ear anatomy affects the motor capabilities of a species, there is still a great deal we can learn about how the species with a rudimentary inner ear system, or no inner ear at all, perceive and respond to motions of the head and body.

13 | CONCLUDING REMARKS

A great deal of research has been invested in understanding how the inner ear develops and how its developmental program has evolved over the course of vertebrate history. This has aided the medical field considerably by providing insights into how mutant variants of human genes in animal models impact both their independent cellular function and the overall function of the inner ear. However, while many genetic factors have already been identified and investigated, there are still gaps in our knowledge concerning all the players involved in otic development and how they interact with other unknown or known factors. Nevertheless, headway has already been made in restoring vestibular function through gene editing tools in some animal models of vestibular diseases. This includes the intraperitoneal injection of antisense oligonucleotides to block the defective cryptic splice site of *Ush1c* or the supplemental knock-in of this gene with synthetic adeno-associated viral vectors to rescue vestibular function in mice models of Usher syndrome (Delmaghani & El-Amraoui, 2020; Lentz et al., 2013; Pan et al., 2017). While it remains to be seen how soon these techniques can be applied to human patients, continuing research into the developmental biology of the inner ear is essential in identifying new targets for this promising area of therapeutics.

CONFLICT OF INTEREST
All authors certify that they have no involvement in or affiliation with any organization or entity with financial or non-financial interests concerning the subject matter discussed in this manuscript.

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ORCID

Kevin H. Yoon https://orcid.org/0000-0002-4699-0471

Andrew J. Waskiewicz https://orcid.org/0000-0002-5306-0643

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