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Zebrafish models of human eye and inner ear diseases

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Abstract

Eye and inner ear diseases are the most common sensory impairments that greatly impact quality of life. Zebrafish have been intensively employed to understand the fundamental mechanisms underlying eye and inner ear development. The zebrafish visual and vestibulo-acoustic systems are very similar to these in humans, and although not yet mature, they are functional by 5 days post-fertilization (dpf). In this chapter, we show how the zebrafish has significantly contributed to the field of biomedical research and how researchers, by establishing disease models and meticulously characterizing their phenotypes, have taken the first steps toward therapies. We review here models for (1) eye...
INTRODUCTION

A total of 285 and 360 million people worldwide are affected with vision and hearing impairments, respectively (World Health Organization, 2015), and the prevalence of these diseases continues to rise in our aging population. The medical, social, and economic impacts of vision and hearing loss (HL) make it imperative to understand the cellular and physiological mechanisms underlying these anomalies. Animal models have already contributed significantly to the field. They are essential to understand what goes wrong in each disease and to develop better diagnostics and treatments.

Zebrafish have proven to be excellent models to study vision and hearing impairment for many reasons. First, the mechanisms of eye and inner ear development are well conserved (Baxendale & Whitfield, 2016; Easter & Nicola, 1996; Gestri, Link, & Neuhauss, 2012; Haddon & Lewis, 1996; Malicki, 1999; Schmitt & Dowling, 1994, 1999; Whitfield, Riley, Chiang, & Phillips, 2002). Second, the anatomical and functional compositions of the zebrafish visual and vestibulo-acoustic systems are similar to that of humans. The zebrafish retina contains the classic vertebrate arrangement of photoreceptors, first- and second-order neurons, and glia, as well as the retinal-pigmented epithelium (RPE) and retinal vasculature (Chhetri, Jacobson, & Gueven, 2014; Malicki, Pooranachandran, Nikolaev, Fang, & Avanesov, 2016). Although zebrafish retinas lack a macula and feature, instead, a highly organized mosaic arrangement of photoreceptors, they are, as in humans, cone-rich and optimized for diurnal activity (Link & Collery, 2015; Raymond et al., 2014). Likewise, despite the lack of a cochlea, the structure of the zebrafish inner ear resembles that of other vertebrates (Baxendale & Whitfield, 2016; Whitfield & Hammond, 2007) and the organization and morphology of the supporting cells and hair cells are also comparable to other vertebrates (Baxendale & Whitfield, 2016; Haddon & Lewis, 1996; Nicolson, 2005). Third, visual and hearing systems develop rapidly and are functional by 5 days post-fertilization (dpf) in zebrafish (Baxendale & Whitfield, 2016; Malicki et al., 2016). Finally, behavioral assays have been developed for visual and hearing function that support rapid assessment of retinal and inner ear functions (Table 1).

Zebrafish are becoming increasingly useful in translational research, contributing to the development of new approaches to understand and treat human diseases (Phillips & Westerfield, 2014). This illustrates the value of this organism as an indispensable model of human diseases. In this chapter, we describe current zebrafish models for vision and hearing impairment. We also review the tools currently available and describe their utility in analyzing defective pathways of vision and hearing.
Table 1  Summary of Behavioral Assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>System</th>
<th>Stage</th>
<th>Measure</th>
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<th>References</th>
</tr>
</thead>
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<tr>
<td>Startle response/Visual motor response</td>
<td>Vision</td>
<td>Larval (from 4 dpf)</td>
<td>Body movement following a change in light intensities</td>
<td>Development and maturation of the visual system</td>
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<tr>
<td>Phototactic behavior</td>
<td>Vision</td>
<td>Larval (from 6 dpf)</td>
<td>Swimming towards perceived light</td>
<td>Light intensity, phototaxis</td>
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<td>Electroretinogram</td>
<td>Vision</td>
<td>Larval (from 4 dpf)</td>
<td>Electrical activity</td>
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<td>Escape response</td>
<td>Vision</td>
<td>Adult</td>
<td>Swimming away from motion</td>
<td>Visual performance and sensitivity</td>
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<td>Auditory-evoked startle response/Touch response</td>
<td>Hearing/Balance</td>
<td>Larval (from 4 dpf) and adult</td>
<td>Swimming away from sound, vibration or touch</td>
<td>Ear and lateral line function</td>
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<td>Rheotactic behavior</td>
<td>Hearing/Balance</td>
<td>Larval and adult</td>
<td>Swimming towards a current</td>
<td>Lateral line function</td>
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<th>Assay</th>
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<tr>
<td>Seeker response</td>
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<td>Larval (from 5 dpf)</td>
<td>Swimming away from seeker</td>
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<td>Vestibulo-ocular reflex</td>
<td>Hearing/Balance</td>
<td>Larval (from 3 dpf) and juvenile</td>
<td>Eye movements following angular/linear acceleration</td>
<td>Vestibular function</td>
<td>Easter and Nicola (1997), Beck, Gilland, Tank, and Baker (2004), Mo et al., 2010, and Bianco et al. (2012)</td>
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<td>Dorsal light reflex</td>
<td>Hearing/Balance</td>
<td>Juvenile and adult</td>
<td>Equilibrium orientation following a change in light position</td>
<td>Vestibular function</td>
<td>Nicolson et al. (1998)</td>
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<td>Body repositioning following dropping and a change in orientation</td>
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<td>Saccular macula microphonic</td>
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<td>Hearing function</td>
<td>Yao et al. (2016)</td>
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1. ZEBRAFISH MODELS OF EYE DISEASE

Forward and reverse genetic approaches using zebrafish models have supplied valuable insights into hereditary eye diseases for several decades. With new and improved tools for genetic manipulation in zebrafish emerging alongside the increased availability of next generation sequencing in the clinical setting, loss-of-function analyses in zebrafish to validate the pathogenicity of candidate alleles identified in human patients have grown in popularity and have made valuable contributions to the global understanding of a wide range of vision disorders (Table 2).

1.1 DISEASES OF PHOTORECEPTORS AND RETINAL-PIGMENTED EPITHELIUM

Retinal degeneration is a leading cause of progressive blindness worldwide. Due to the extensive molecular interplay between the photoreceptors in the neural retina and the RPE, it is not surprising that mutations in genes that function in either cell type can lead to a common end result of vision loss due to photoreceptor dysfunction and death. Zebrafish models of loci that contribute to dysfunction of RPE or photoreceptors have increased our understanding of the genes and genetic pathways underlying human disease, providing necessary insights for the development of improved diagnostic tools and therapeutic interventions.

Genes that regulate the highly conserved process of phototransduction, whereby photon-activated visual pigment molecules in the photoreceptor outer segments initiate G-protein coupled signaling, are common causes of inherited retinal degeneration (Yau & Hardie, 2009), as are factors involved in the visual cycle that converts 11-cis-retinal to all-trans-retinal and back again (Baehr, Wu, Bird, & Palczewski, 2003).

Zebrafish models of achromatopsia, a cone-rod dystrophy caused by mutations in PDE6C, have made significant contributions to our understanding of this disorder. PDE6C encodes a cone-specific phosphodiesterase, an enzyme required for regulation of cGMP activity in photoreceptors. Rod degeneration subsequent to the loss of cones indicates a non-cell autonomous action, termed the “bystander effect”, which is a variable but common outcome in retinal degenerative diseases with primary effects in either rods or cones.

Stearns, Evangelista, Fadool, and Brockerhoff (2007) identified a zebrafish pde6c mutant showing a marked cone degeneration phenotype at 3 dpf, followed by corresponding rod death. Taking advantage of developmental timing in the zebrafish retina, where cone maturation precedes that of rods, Stearns et al. (2007) were able to illustrate that cone:rod density ratios were a critical element of the degenerative pattern. Their data demonstrated that rods in cone-rich regions of the developing retina, and in established central regions of the mature retina, were more susceptible to degeneration than rods occupying regions of higher rod density at the retinal margins in developing and mature, regenerating zebrafish retinas.
### Table 2 Zebrafish Models of Eye Disease

<table>
<thead>
<tr>
<th>Eye Disorder</th>
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<th>Origin of Zebrafish Model (s)</th>
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<td>CRYAA</td>
<td>TALENs</td>
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<td></td>
<td>123580</td>
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<td>Greenlees et al. (2015)</td>
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<td>616655</td>
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<td>ENU</td>
<td>Krock et al. (2007) and</td>
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<td>300390</td>
<td></td>
<td>Moosajee et al. (2008, 2009, 2016)</td>
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<td>FZD5</td>
<td>MO</td>
<td>C. Liu et al. (2016)</td>
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<td></td>
<td>601723</td>
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<td></td>
<td>YAP</td>
<td>ENU</td>
<td>Miesfeld et al. (2015)</td>
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<td>606608</td>
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<tr>
<td>Cone-rod dystrophies</td>
<td>GNAT2</td>
<td>ENU</td>
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<td>139340</td>
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<td>Kennedy et al. (2007)</td>
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<td>GUCY2D</td>
<td>MO</td>
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<td>PDE6C</td>
<td>ENU</td>
<td>Stearns et al. (2007) and</td>
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<td>600827</td>
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<td>Viringipurampeer et al. (2014)</td>
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<td>RAX</td>
<td>MO</td>
<td>Nelson, Park, and Stenkamp (2009)</td>
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<td>610362</td>
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<td></td>
<td>UNC119</td>
<td>MO</td>
<td>Wright et al. (2011) and</td>
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<td>604011</td>
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<td>Rainy et al. (2016)</td>
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<td></td>
<td>CACNA1F</td>
<td>ENU</td>
<td>Jia et al. (2014)</td>
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<td>GPR179</td>
<td>MO</td>
<td>Peachey et al. (2012)</td>
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<td>614515</td>
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<td></td>
<td>GRM6</td>
<td>MO</td>
<td>Huang, Haug, Gesemann, and Neuhauss (2012)</td>
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<td>604096</td>
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<td>NYX</td>
<td>MO</td>
<td>Bahadori et al. (2008) and</td>
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<td>300278</td>
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<td>Peachey et al. (2012)</td>
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<td></td>
<td>ZNF408</td>
<td>MO</td>
<td>Collin et al. (2013)</td>
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<td></td>
<td>616454</td>
<td></td>
<td></td>
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<tr>
<td>Exudative vitreoretinopathy</td>
<td>FOXC1</td>
<td>MO</td>
<td>Skarie and Link (2009)</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>601090</td>
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<td></td>
<td>OPTN</td>
<td>ENU</td>
<td>Paulus and Link (2014)</td>
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<td></td>
<td>602432</td>
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<td></td>
<td>WDR36</td>
<td>MO</td>
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### Table 2 Zebrafish Models of Eye Disease—cont’d

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<td>Chao et al. (2010)</td>
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<td>ARL6 613575</td>
<td>MO</td>
<td>Pretorius et al. (2010)</td>
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<td></td>
<td>C2ORF71 613425</td>
<td>MO</td>
<td>Nishimura et al. (2010) and Y. P. Liu et al. (2016)</td>
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<td></td>
<td>CERKL 608381</td>
<td>MO</td>
<td>Riera, Burguera, Garcia-Fernandez, and Gonzalez-Duarte (2013) and Li et al. (2014)</td>
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<td></td>
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<td>TALEN, INS</td>
<td>Lee, Wallingford, and Gross (2014) and Soens et al. (2016)</td>
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<td>MO</td>
<td>Nishiguchi et al. (2013)</td>
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<td>PRPF4 607795</td>
<td>MO</td>
<td>Linder et al. (2011, 2014)</td>
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<td>PRPF31 606419</td>
<td>MO</td>
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<td>MO</td>
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<td></td>
<td>SNRNP200 601664</td>
<td>MO</td>
<td>Y. Liu et al. (2015)</td>
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</table>
More recently, Viringipurampeer et al. (2014), reported that cell death in pde6d mutants was the result of necroptotic pathway activation, demonstrated by their success in rescuing photoreceptor death by morpholino (MO) knockdown of rip3, a key regulator of necroptosis. Taken together, these studies offer key insights into the underlying causes of photoreceptor degeneration in PDE6D achromatopsia as well as providing potential avenues for developing treatments.

Choroideremia is a rare, X-linked form of retinal degeneration caused by mutations in CHM, which encodes the Rab-escort protein REP1. REP1 is a key component of a catalytic complex in the RPE, Rab GG transferase II, that regulates Rab-mediated melanosome trafficking and phagocytosis. In the absence of functional Rep1, posttranslational modifications of Rab are affected, leading to degeneration of the RPE and the vascular network known as the choroid that overlies the RPE. The zebrafish chm mutant has previously provided significant data on the molecular and cellular changes involved in choroideremia (Krock, Bilotta, & Perkins, 2007; Moosajee, Gregory-Evans, Ellis, Seabra, & Gregory-Evans, 2008) and is now contributing to preclinical trials for aminoglycoside drug therapy. Previous studies of chm provided Moosajee et al. (2016) with an ideal system in which to demonstrate that nonsense suppression agents could successfully repress retinal cell death and restore biochemical function of chm in vivo, bringing this treatment one step closer to clinical trial approval for CHM patients.

1.2 ZEBRAFISH TOOLS FOR DIAGNOSTIC MEDICINE

In addition to testing the pathogenicity of new variants of individual disease genes, zebrafish models have also provided crucial evidence for genetic interactions between previously identified monogenic disorders. More than 50 genes to date have
been linked to retinitis pigmentosa (RP), a common form of progressive vision loss due to retinal degeneration. A number of nonsyndromic RP genes (https://sph.uth.edu/retnet/sum-dis.htm) are also involved in syndromic disorders that include RP (Table 2). Two genes, C2ORF71 and RP1L1, both previously known to cause autosomal recessive RP, were recently implicated in a digenic form of syndromic RP, suggesting hitherto unknown haploinsufficiency at both loci (Y.P. Liu et al., 2016). Zebrafish MO and clustered regularly interspaced short palindromic repeats (CRISPRs) models recapitulated the additive loss-of-function effect noted in the retinas and brains of digenic patients, thus validating the rare genetic presentation and providing a new diagnostic option for undiagnosed syndromic RP patients going forward.

Beyond the currently identified disease genes, phenotypic models derived from forward mutagenesis screens as well as reverse genetic approaches targeting components of eye development and function have enhanced the field of eye research tremendously. The avascular zebrafish mutant cloche has been a heavily utilized model of vascular development since its discovery (Stainier, Weinstein, Detrich, Zon, & Fishman, 1995), and eye phenotypes resulting from affected blood vessel formation have contributed to better understanding of the role of vascularization in retinal and lens development (Dhakal et al., 2015; Goishi et al., 2006). Recently, cloche has been identified molecularly as the bHLH-PAS transcription factor npas4l (Reischauer et al., 2016), which has yet to be linked to any type of human disease, but can now be added to screening protocols for vascular disorders of the retina and other systems.

There is a strong precedent for investigations into genetic mechanisms preceding the identification of a gene as causative of human disease. For example, noting that several types of RP are caused by ubiquitously expressed mRNA splicing factors, Linder et al. (2011) and Ruzickova and Stanek (2016) conducted experiments to illuminate the cell-specific effect of these mutations. In this study, they also reported a retina-specific phenotype caused by depletion of another member of the tri-snRNP family, prfp4, which had not been previously identified as a human disease gene. Several years later, PRFP4 mutations were verified as causative of RP (Chen et al., 2014; Linder et al., 2014).

2. ZEBRAFISH MODELS OF EAR DISEASE

A significant portion of the genetic mutations linked to sensorineural deafness are allelic to mutations that cause syndromic disorders. Additionally, numerous genes have been associated with nonsyndromic deafness (Table 3), and their identification has been significantly facilitated by zebrafish research. Zebrafish models of hair cell dysfunction exhibit easily scorable behavioral traits (Table 1), providing an accessible and reproducible method to evaluate loss of function.

Clinically, HL is the most common reported birth defect in developed countries (Hilgert, Smith, & Van Camp, 2009). This condition can have a conductive (outer and/or middle ear), sensorineural (inner ear), mixed (outer, middle, and inner ear), or central auditory origin (Smith, Shearer, Hildebrand, & Van Camp, 1993). Clinical
<table>
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<tr>
<th>Nonsyndromic Ear Disorder</th>
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<td>Söllner et al. (2004) and Blanco-Sanchez et al. (2014)</td>
</tr>
<tr>
<td>DFNB18A</td>
<td>USH1C 605242</td>
<td>MO, ENU</td>
<td>Phillips et al. (2011)¹² and Blanco-Sanchez et al. (2014)²</td>
</tr>
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<td>ENU</td>
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<td>ILDR1 609739</td>
<td>MO</td>
<td>Sang et al. (2014)</td>
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<tr>
<td>DFNB48</td>
<td>CIB2 605564</td>
<td>MO</td>
<td>Riazuddin et al. (2012)</td>
</tr>
</tbody>
</table>
manifestation can occur before (prelingual) or after (postlingual) the onset of speech (Smith et al., 1993). Whereas congenital deafness is considered prelingual, not all prelingual deafness is congenital (Smith et al., 1993).

HL prevalence increases as the population ages. It has been estimated that 1 in 500 newborns is affected by moderate to profound bilateral sensorineural hearing loss (SNHL) (Morton & Nance, 2006). However, HL prevalence increases to 3.5 in 1000 by adolescence, showing the relevant significance of postlingual SNHL (Morton & Nance, 2006).

Vestibular disorders can originate in the cerebral cortex (central) or at the level of the eye and/or inner ear (peripheral). Their estimated occurrence is more variable and less accurate than HL, due to difficulty in the diagnosis. As an example, the lack of a neonatal standardized test to measure vestibular function prevents calculation of the congenital incidence. One vestibular epidemiological study projected that, in the United States 35% of adults over 40 have been affected by balance disorders (Agrawal, Carey, Della Santina, Schubert, & Minor, 2009). As with HL prevalence, the frequency of vestibular disorders increases with age, and an estimated 80% of the population over 65 have experienced vestibular dysfunction of some kind (Zalewski, 2015).

Table 3 Zebrafish Models of ear Disease—cont’d

<table>
<thead>
<tr>
<th>Nonsyndromic Ear Disorder</th>
<th>Human Gene/OMIM#</th>
<th>Origin of Zebrafish Model</th>
<th>References</th>
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<tr>
<td>DFNB66</td>
<td>DCDC2, 610212</td>
<td>MO</td>
<td>Grati et al. (2015)</td>
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<td>S1PR2, 610419</td>
<td>MO, ENU</td>
<td>Hu et al. (2013) and Santos-Cortez et al. (2016)</td>
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<td>DFNB84B</td>
<td>OTOGL, 614925</td>
<td>MO</td>
<td>Yariz et al. (2012)</td>
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<td>TMEM132E, 616178</td>
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<td>MO</td>
<td>Diaz-Horta et al. (2014)</td>
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<td>DFNB105</td>
<td>CDC14A, 616958</td>
<td>MO</td>
<td>Delmaghani et al. (2016)</td>
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</table>

Genes represented in the tables were identified by cross-referencing searches in ZFIN (http://zfin.org), the Online Mendelian Inheritance in Man database (www.omim.org), and PubMed (http://www.ncbi.nlm.nih.gov/pubmed). Criteria for inclusion were restricted to zebrafish models of genes associated with human diseases affecting vision and/or hearing for which ocular or otic phenotypes were described. The following numbers indicate the method used to generate the zebrafish model for a given disease. 1, MO, morpholino; 2, ENU, N-ethyl-N-nitrosourea; 3, TALENs, transcription-activator like effector nucleases; 4, CRISPR, clustered regularly interspaced short palindromic repeat; 5, Viral insertion.
Current treatment for SNHL varies according to the severity and age of onset. For severe to profound prelingual disorders, early intervention with cochlear implants yields the best results (Connor, Craig, Raudenbush, Heavner, & Zwolan, 2006; Connor, Hieber, Arts, & Zwolan, 2000; James, Rajput, Brinton, & Goswami, 2008). Treatments for vestibular disorders differ according to the specific diagnosis and include dietary changes, physical therapy, and pharmaceutical and surgical approaches (Driscoll, Kasperbauer, Facer, Harner, & Beatty, 1997; Hain & Yacovino, 2005; Herreraiz et al., 2010; McClure, Lycz, & Baskerville, 1982; Ruckenstein, Rutka, & Hawke, 1991; Smith, Sankar, & Pfleiderer, 2005; Strupp et al., 2008, 2011, 2004; Takeda, Morita, Hasegawa, Kubo, & Matsunaga, 1989; Torok, 1977; Whitney & Rossi, 2000). Like the disorders themselves, the success rates of these treatments are highly variable. Moreover, the impact of early childhood vestibular dysfunction has been underestimated, although it can hamper development of motor skills and even literacy due to poor gaze stability (Casselbrant, Villardo, & Mandel, 2008; Christy, Payne, Azuero, & Formby, 2014; Cushing, Papsin, Rutka, James, & Gordon, 2008; Janky & Givens, 2015; Li, Hoffman, Ward, Cohen, & Rine, 2016; Rine, 2009; Rine et al., 2000; Rine, Dannenbaum, Szabo, 2016; Rine, Spielholz, Buchman, 2001; Rine & Wiener-Vacher, 2013; Wiener-Vacher, Ledebe, & Bril, 1996).

Finding and understanding the etiology of human ear diseases are essential for improving and extending therapy success, proposing novel approaches, and finding cures. Due to its accessibility, rapid development, and molecular and genetic manipulations, the zebrafish inner ear is an ideal model to reach these goals as well as to study sensorineural inner ear dysfunction (Table 3). In this section, we discuss the contribution of zebrafish models of genetic inner ear diseases to validation of candidate genes and elucidation of pathogenesis.

2.1 INNER EAR DISEASE

Historically, based on their mode of inheritance, loci associated with nonsyndromic deafness have been grouped into four distinct DFN categories. Loci associated with an autosomal dominant pattern are grouped into the DFNA class, those with an autosomal recessive mode of inheritance are classified as DFNB, and those associated with sex chromosomes are called DFNX and DFNY (Smith et al., 1993). Genetically, such categorization implies that, depending on the mutation, the same gene can be binned into both DFNA and DFNB categories or even be associated with syndromic HL.

Inner ear research has made considerable progress in identifying genes involved in hearing and balance. In 1996, POU3F4 was the only gene associated with human deafness and 10 loci were linked to each DFNA and DFNB category (Petit, 1996). Similarly, only five genes (MITF, PAX3, EDNRB, EDN3, and MYO7) were linked to syndromic human deafness (Petit, 1996). During that same year, the Boston and Tübingen forward genetic screens in zebrafish identified 33 genes involved in ear development (Malicki et al., 1996; Whitfield et al., 1996). These genes affect
specification of the otic placode, early morphogenesis of the vesicle, patterning, development of otoliths and semicircular canals, or ear size. The Tübingen screen also identified mutants with vestibular dysfunction despite having morphologically normal ear structures, as well as mutants with developmental anomalies in the ear and in other tissues resembling human syndromic conditions (Nicolson et al., 1998; Whitfield et al., 1996). Currently, 141 DFN loci are known, and, using both forward and reverse genetic approaches, zebrafish models provide a strong experimental platform for testing and validating candidate genes implicated in otic pathologies.

2.1.1 Sensorineural syndromic deafness
Currently, approximately 400 human syndromes associated with HL have been described (Toriello, Reardon, & Gorlin, 2004). In this section, we present two zebrafish models of SNHL for Branchiootorenal (BOR) and Waardenburg syndromes. Zebrafish models of syndromic eye and inner ear diseases are discussed in section 4 of this chapter.

2.1.1.1 Branchiootorenal/Branchiootic syndrome
*EYA1* and *SIX1* encode evolutionary conserved transcriptional cofactors that assemble into a transcriptional unit through direct physical interaction of the SIX and EYA domains (Buller, Xu, Marquis, Schwanke, & Xu, 2001; Grifone et al., 2004; Ikeda, Watanabe, Ohto, & Kawakami, 2002; Ozaki, Watanabe, Ikeda, & Kawakami, 2002; Pignoni et al., 1997; Ruf et al., 2004). In this transcriptional complex, SIX1 provides the DNA binding activity through its homeodomain and EYA1 confers transcriptional activation. In humans, mutations in *EYA1* and *SIX1* result in the autosomal dominant genetic disorders BOR and Branchiootic (BO) syndromes (Abdelhak et al., 1997; Buller et al., 2001; Ozaki et al., 2002; Ruf et al., 2004). BOR/BO patients suffer moderate to severe conductive, sensorineural or mixed HL, branchial fistulae, and variable renal dysfunction. Other symptoms include vestibular aqueduct dilation, arrested development of the cochlea, vestibular canals, and facial nerve, cleft palate and cataracts (Ruf et al., 2004; Sanggaard et al., 2007).

In vertebrates, these genes are expressed in the preplacodal domain (PPD) (Sahly, Andermann, & Petit, 1999; Sato et al., 2010), an anteriorly located horseshoe shaped embryonic field established by the end of gastrulation (Schlosser & Ahrens, 2004). The PPD lies between the neural and non-neural ectodermal borders and partially overlaps with the anterior neural crest progenitor domain. During development, preplacodal cells segregate and sort into subdomains that give rise to the otic, trigeminal, and nasal placodes and the lens (Saint-Jeannet & Moody, 2014). Cranial clinical features of BOR patients correspond to the predicted PPD derivatives and the common embryonic origin shared by preplacodal and anterior neural crest precursors.

In zebrafish, *eya1* and *six1* expression initiates around 9–10 hpf (Bessarab, Chong, & Korzh, 2004; Sahly et al., 1999). Both genes are expressed throughout
the otic preplacodal and placodal tissues (Kozlowski, Whitfield, Hukriede, Lam, & Weinberg, 2005). However, their expression is restricted to the ventral half of the otic vesicle by 24 hpf. In 1996, three mutant alleles of the eyal (dog-eared) gene were identified that truncate the protein at the Eya domain, thus disrupting assembly of the Eyal-Six1 transcriptional unit (Whitfield et al., 1996). In the eyal zebrafish mutant at 24 hpf, the otic vesicle is present with no apparent morphological defects. Development of the semicircular canals initiates by 48 hpf in zebrafish, when epithelial projections evaginate from the vesicle walls toward the lumen (Whitfield et al., 2002). At this stage, eyal mutants develop a smaller otic vesicle with abnormal morphogenesis of the semicircular canals (Kozlowski et al., 2005; Whitfield et al., 1996). As a result, the inner ear is severely dysmorphic. Additional analysis showed that Eyal is necessary for promoting the induction and delamination of neuronal precursors that give rise to the statoacoustic ganglion (SAG), as well as hair cell survival (Kozlowski et al., 2005; Whitfield et al., 2002).

In zebrafish, Six1 function has been studied by antisense MO oligonucleotides (Bricaud & Collazo, 2006, 2011). The six1 morphant shows neuronal lineage defects opposite to that of eyal, indicating that Six1 inhibits neuronal specification. Specifically, Bricaud & Collazo (2011) overexpressed a mutant form of Six1 that abrogates its interaction with Eyal without affecting its DNA binding or ability to form a transcriptional repressor complex with Groucho1. The number of SAG precursor cells was reduced, similar to six1 morphants. This suggested that Eyal and Six1 do not cooperate physically to specify the neuronal lineage and that they have both dependent and independent roles during development. These zebrafish models of BOR/BO syndromes have demonstrated that mutations in eyal or six1 are not equivalent and that these two genes differentially affect otic development. Thus, BOR and BO have distinct cellular etiologies, meaning that human patients may need distinct therapeutic approaches depending on their genotype.

2.1.1.2 Waardenburg syndrome types IIE (WS2E) and IV (WS4)
SOX10 is another key gene in otic development. In humans, mutations in this transcription factor lead to Waardenburg syndrome types IIE and IV. This genetic condition causes SNHL, variable Hirschsprung disease, and pigmentation defects, and it can have a recessive or dominant inheritance pattern (Kuhlbrodt et al., 1998; Potterf, Furumura, Dunn, Arnheiter, & Pavan, 2000).

During zebrafish development, sox10 is expressed in the posterior region of the PPD, the anterior neural crest progenitor domain, and the entire otic placode and vesicle (Dutton et al., 2009). By 24 hpf, the otic vesicle of the sox10 (colourless, cls) mutant has no apparent morphological defects (Dutton et al., 2009). However, by 48 hpf, the cls otic vesicle is smaller, and development of the semicircular canals is severely affected. The mutant phenotype is variable and ranges from small to swollen ears with or without distinguishable semicircular canal pillars (Dutton et al., 2009). Molecular analysis showed that Sox10 is necessary for correct inner ear patterning, proper macular development, and expression of gap junction coding genes such as connexin (cx) 33.8 and connexin 27.5 (Dutton et al., 2009). Prior to the
published zebrafish work in 2009, it was proposed that Waardenburg syndrome IIE and IV forms of deafness were due to a deficient contribution of melanocytes to the stria vascularis of the cochlea causing dysregulation of the endolymph ion composition (Matsushima et al., 2002; Tachibana et al., 1992, 2003). However, the zebrafish sox10 otic expression pattern and mutant phenotype indicated a more complex etiology in which otic patterning, epithelial integrity, and possible compartmentalization play roles in Waardenburg syndromes IIE and IV (Dutton et al., 2009). In 2013, a more detailed evaluation of 14 patients with mutations in SOX10 showed that all were affected by agenesis or hypoplasia of one or more semicircular canals, enlarged vestibule, reduced cochlear size without compartmentalization defects, and variable defects at the level of cochlear nerve (Elmaleh-Berges et al., 2013), phenotypes predicted from the zebrafish model.

2.1.2 Nonsyndromic deafness

To validate candidate genes involved in nonsyndromic deafness, zebrafish researchers have adopted both forward and reverse genetic approaches based on loss-of-function mutations using N-ethyl-N-nitrosourea (ENU)-based mutagenesis, MO, viral insertions, or genome editing techniques like transcription-activator like effector nuclease (TALEN) or CRISPR. Some of these models lack obvious morphological defects but others show broad morphological impacts. Based on phenotypes, predicted proteins, and proposed biological functions or requirement, we have established the following categories of zebrafish models.

2.1.2.1 Epithelial integrity

DFNA28 is a form of autosomal dominant deafness. Zebrafish dfna28 (grhl2b) encodes a transcription factor present in the otic vesicle at 24 hpf (Han et al., 2011). It is involved in positive regulation of claudin b and epcam that both encode components of the apical tight junction complex (Han et al., 2011). Zebrafish homozygous mutants have abnormal swimming behavior characterized by a corkscrew pattern and an increased latency to respond to sound (Han et al., 2011). grhl2b mutation affects both otoliths and semicircular canals. By 5 dpf, mutant semicircular canals form but present localized epithelial dentations. Unlike eya1 or sox10 mutants, grhl2b mutants lack severe patterning defects and develop normal SAG and hair cells (Han et al., 2011). Because grhl2b is necessary for epithelial integrity, the authors proposed that vestibular dysfunction is due to a leaky epithelial barrier that leads to dysregulation of endolymph composition.

ILDR1 encodes a transmembrane protein associated with tight junction complex proteins, and mutations in this gene give rise to DFNB42 (Borck et al., 2011). The zebrafish idlr1b MO model has semicircular canal and lateral line migration defects (Sang et al., 2014). Interestingly, the authors also showed that this transmembrane protein is necessary for the expression of atp1b2b, a gene encoding a Na⁺/K⁺ ATPase transporter previously shown to promote morphogenesis of the semicircular canal, thus providing potential insight into the etiology of the disease.
Two other genes have been implicated in semicircular canal morphogenesis based on MO studies. The first, \textit{dfna5b}, an ortholog of \textit{DFNA5}, is thought to act as a transcription factor required for expression of enzymes involved in synthesis of extracellular matrix (Busch-Nentwich, Sollner, Roehl, & Nicolson, 2004). \textit{dfna5b} mRNA is detected in the otic vesicle at the columnar epithelial projections that give rise to the semicircular canals (Busch-Nentwich et al., 2004). The second gene, \textit{tmie}, encodes the transmembrane protein Tmie (Dfnb6). \textit{tmie} morphants show no gross patterning defects but exhibit a reproducible failure in the epithelial fusion between the ventral bulge and projection (Shen et al., 2008). These zebrafish mutants have severe (\textit{tmie}) or mild (\textit{dfna5b}) mechanosensory dysfunction, and neither develops gross malformation of the balance organ (Gleason et al., 2009; Varshney et al., 2015).

2.1.2.2 Sensory organ architectural defects

The glycoproteins Otogelin (\textit{OTOG}), Otogelin-like, alpha-Tectorin (\textit{TECTA}), beta-Tectorin, and Otolin are components of the otolith membrane in fish and the otoconial and tectorial membranes in mammals (Deans, Peterson, & Wong, 2010; Goodyear & Richardson, 2002; Yariz et al., 2012). These acellular membranes transmit mechanical stimuli to the hair cell mechanoreceptor (Eatock, Fay, & Popper, 2006; Richardson, de Monvel, & Petit, 2011; Tavazzani et al., 2016). In humans, mutations in \textit{OTOG} have been associated with deafness (DFNB18b) and balance defects, whereas mutations in \textit{TECTA} have been linked to recessive (DFNB21) and dominant (DFNA8/12) forms of deafness (Meyer et al., 2007; Mustapha et al., 1999; Naz et al., 2003; Schraders et al., 2012; Verhoeven et al., 1997).

Genetic analysis of the \textit{otogelin} (\textit{einstein}) and \textit{tecta} (\textit{rolling stone}) zebrafish mutants revealed that these two extracellular proteins act independently and in a sequential mechanism (Stooke-Vaughan, Obholzer, Baxendale, Megason, & Whitfield, 2015). Otogelin plays the initial role in the otolith seeding process, and alpha-Tectorin then functions to maintain tethering. Interestingly, \textit{otogelin} zebrafish mutants have aberrant swimming behavior probably because they develop only one otolith that often tethers at the saccular macula (Stooke-Vaughan et al., 2015; Whitfield et al., 1996). In contrast, \textit{tecta} zebrafish mutants swim normally and have two otolites, although the saccular otolith tends to be dislodged (Stooke-Vaughan et al., 2015; Whitfield et al., 1996). Double \textit{otogelin};\textit{tecta} zebrafish mutants have an additive phenotype; only one otolith forms, and it fails to tether (Stooke-Vaughan et al., 2015).

These zebrafish studies not only provide models for otoconia-associated vestibular dysfunction, like benign paroxysmal positional vertigo, but also contribute to our understanding of the mechanisms of extracellular matrix synthesis and remodeling.

2.1.2.3 Hair cell dysfunction

Mechanoreceptor structural defects and impaired neurotransmission severely affect hearing and balance (Blanco-Sanchez, Clement, Fierro, Washbourne, & Westerfield, 2014; El-Amraoui & Petit, 2005; Ernest et al., 2000; Gopal et al., 2015; Phillips et al., 2011; Reiners, Nagel-Wolfrum, Jurgens, Marker, & Wolfrum, 2006; Seiler
et al., 2005; Söllner et al., 2004). In this section, we discuss zebrafish models of mechanoreceptor and synaptic dysfunction.

2.1.2.3.1 Mechanoreceptor dysfunction
In humans, mutations in MYO7AA (DFNB2, DFNA11, USH1B), USH1C (DFN18A), CDH23 (DFNB12, USH1D), PCDH15A (DFNB23, USH1F) and CLRN1 (USH3A) genes can give rise to Usher syndrome (USH) or nonsyndromic forms of deafness or RP. These genes encode a variety of proteins such as atypical myosin motor, scaffold, adhesion, and transmembrane molecules that assemble into complexes (Reiners et al., 2006). Mechanoreceptors of zebrafish models for these genes are characterized by the presence of splayed hair bundles, consistent with the role of these proteins in forming the connecting links between stereocilia and kinocilia (Blanco-Sanchez et al., 2014; Ernest et al., 2000; Gopal et al., 2015; Phillips et al., 2011; Seiler et al., 2005; Söllner et al., 2004). Work from our group showed that in the hair cells, harmonin, the product of the ush1c gene, Cdh23, and Myo7aa assemble into a complex at the level of the endoplasmic reticulum (ER) (Blanco-Sanchez et al., 2014). Disruption in the assembly affects the homeostasis of the secretory pathway, induces the ER stress response, and promotes cell death through the CDK5 signaling pathway.

The zebrafish myo6b mutant is a model for DFNA22 and DFNB37 forms of deafness. Myo6b is another atypical myosin that plays a dual role as a transporter or an anchor depending on ATP concentration (De La Cruz, Ostap, & Sweeney, 2001; Wells et al., 1999). Myo6b has been associated with apical vesicular trafficking, and myo6b mutations impair stereocilia growth and result in variable splaying of the hair bundle or occasional stereociliary fusion (Seiler et al., 2004). Detachment of the plasma membrane from the cuticular plate in the apical region and vesicle accumulation have also been reported in the myo6b mutant.

Recent MO-based studies in zebrafish have linked the function of cib2 (DFNB48, USH1J, fam65b (DFNB104), tmem132e (DFNB99), dcdc2 (DFN66) and cdc14a (DFNB105) with mechanoreceptor development and/or function (Delmaghani et al., 2016; Diaz-Horta et al., 2014; Grati et al., 2015; Li et al., 2015; Riazuddin et al., 2012).

2.1.2.3.2 Synapse dysfunction
In humans, mutations in SLC17A8 and OTOFERLIN (OTOF) cause DFNA25 and DFNB9, respectively (Adato, Raskin, Petit, & Bonne-Tamir, 2000; Migliosi et al., 2002; Ruel et al., 2008; Yasunaga et al., 2000). SLC17A8 is a glutamate vesicular transporter and OTOF is a six C-2 domain transmembrane protein postulated to promote vesicular fusion at the presynaptic membrane (Chatterjee et al., 2015; Obholzer et al., 2008; Takamori, Malherbe, Broger, & Jahn, 2002; Yasunaga et al., 1999). Zebrafish has two otof copies (otofa and otobuf) that are expressed in a complementary manner at the level of the sensory patches and one copy of slc17a8 that is expressed in hair cells (Chatterjee et al., 2015; Obholzer et al., 2008). As expected, all three proteins are enriched at the basolateral membrane
slc17a8 zebrafish mutants show the circler phenotype characteristic of other inner ear mutants, i.e., no startle response to dish-tapping, vestibular areflexia, and a corkscrew swimming pattern (Obholzer et al., 2008) (Table 1). In zebrafish models of DFNB9, vestibular defects are present only when both otof copies are knocked down with MOs (Chatterjee et al., 2015). In both models, mechanotransduction is unaffected as assayed by uptake of FM1-43 or YO-PRO1 dyes. Action currents in postsynaptic acousticolateralis neurons were absent in slc17a8 mutants, indicating a failure in neurotransmission (Obholzer et al., 2008).

3. ZEBRAFISH MODELS OF SYNDROMES AFFECTING EYE AND/OR EAR
3.1 USHER SYNDROME

Mutations in a number of genes are known to affect both eye and inner ear function in humans. Zebrafish models of combined eye and inner ear disease can help identify common and single pathological mechanisms involved in the dysfunction of each organ. In this section, we discuss zebrafish models of human diseases in which both eye and ear function are affected.

Zebrafish has recently emerged as an excellent model for studies of USH. Not only can visual and vestibular function be quickly assayed (Table 1), but also the tissues of interest are accessible for live imaging and studies at cellular resolution (Blanco-Sanchez et al., 2014; Ebermann et al., 2010; Glover, Mueller, Sollner, Neuhauss, & Nicolson, 2012; Gopal et al., 2015; Ogun & Zallocchi, 2014; Phillips et al., 2011; Seiler et al., 2005).

USH is the leading cause of hereditary combined deafness and blindness, affecting 1 in 6000 Americans. It is characterized by congenital sensorineural deafness, RP, and, in some cases, balance problems (Mathur & Yang, 2015). Three clinical types (USH1—USH3) are distinguished by symptom onset and severity. USH1 is the most clinically severe with profound congenital deafness, vestibular dysfunction and an onset of retinal degeneration in early childhood. USH2 is the most common subtype and is clinically distinct from USH1 manifestations due to hearing impairment in the moderate to severe range, a later onset of vision loss in the second decade of life, and the absence of vestibular symptoms. USH3 is the most variable subtype with stable progressive or profound HL, variable vestibular dysfunction, and progressive vision loss. In some cases, mutations in genes that cause USH can also lead to RP or nonsyndromic deafness depending on their nature and position in the gene of interest (Tables 2—4). Here, we describe zebrafish models of USH syndrome.

3.1.1 USH1C

The USH1C gene encodes a key organizer scaffold protein, harmonin, that binds multiple proteins and organizes them into a complex. The USH proteins are among
Table 4  Zebrafish Models of Syndromes Affecting Eye and/or Ear

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Human Gene/OMIM#</th>
<th>Differential Diagnosis</th>
<th>Zebrafish Model Phenotype</th>
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<td>Zebrafish Models of Syndromes Affecting Eye and/or Ear</td>
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<tr>
<td>Bardet–Biedl syndrome (BBS)</td>
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<td></td>
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<td>BBS5</td>
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<td>Al-Hamed et al. (2014)</td>
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<td></td>
<td>C8ORF37 614477</td>
<td>BBS21, RP, cone-rod dystrophy</td>
<td>Impaired visual behavior</td>
<td>Heon et al. (2016)</td>
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<td></td>
<td>CEP290 610142</td>
<td>BBS, LCA, Meckel syndrome, NPHP</td>
<td>Disorganized photoreceptors, reduced visual function</td>
<td>Baye et al. (2011)</td>
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<td></td>
<td>IFT172 607386</td>
<td>BBS, RP71, short-rib thoracic dysplasia 10 with or without polydactyly</td>
<td>Disorganized photoreceptors, thin outer nuclear layer, retinal degeneration</td>
<td>Lunt, Haynes, and Perkins (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reduced otic vesicle, kinocilia defects</td>
<td></td>
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<td>Branchiootic syndrome (BO/BOR)</td>
<td>EYA1 601653</td>
<td>BO syndrome, BOR syndrome</td>
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<td>BO3, DFNA23</td>
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<td>CHARGE syndrome</td>
<td>CHD7 608892</td>
<td>CHARGE syndrome</td>
<td>Retinal development defects</td>
<td>Bricaud and Collazo (2006)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Abnormal otoliths</td>
<td>Patten et al. (2012), Balow et al. (2013), and Balasubramanian et al. (2014)</td>
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Table 4  Zebrafish Models of Syndromes Affecting Eye and/or Ear—cont’d

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Human Gene/OMIM#</th>
<th>Differential Diagnosis</th>
<th>Zebrafish Model Phenotype</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Jalili syndrome              | CNNM4 607805    | Jalili syndrome                               | Reduction of retinal ganglion cell numbers                                              | Polok et al. (2009)
| Joubert syndrome (JBTS)      | AHI1 608894     | JBTS                                          | Microphthalmia, photoreceptor cilia defects                                              | Simms et al. (2012)
|                              |                 |                                               | Retinal degeneration                                                                    | Song, Dudinsky, Fogerty, Galvin, and Perkins (2016)
|                              | ARL13B 608922   | JBTS                                          | Photoreceptor outer segment defects                                                     | Owens et al. (2008)
|                              |                 |                                               | Hair cell number                                                                        | and Bachmann-Gagescu et al. (2011)
|                              | CC2D2A 612013   | JBTS, Meckel syndrome, COACH syndrome         | Microphthalmia                                                                          | Lee et al. (2012)
|                              |                 |                                               | Abnormal otoliths                                                                       | Luo, Lu, and Sun (2012)
|                              | CEP41 610523    | JBTS                                          | Microphthalmia                                                                          | Thomas et al. (2014)
|                              | INPP5E 613037   | JBTS                                          | Microphthalmia, disorganized retina                                                     | Beck et al. (2014)
|                              | PDE6D 602676    | JBTS                                          | Disorganized retina, microphthalmia                                                     | and Roosing et al. (2014)
|                              | POC1B 614784    | JBTS, LCA                                     | Microphthalmia, affected retinal cilia, reduced visual function                        | Shen and Raymond (2004)
| Leber congenital amaurosis   | CRX 602225      | LCA, cone-rod dystrophy                       | Retinal dysmorphogenesis, retinal cell death                                            | Asai-Coakwell et al. (2007, 2013), Valdivia et al. (2016), and Gosse and Baier (2009)
<p>| (LCA)                        | GDF6 601147     | LCA, Klippel–Feil syndrome 1, microphthalmia with coloboma 6 | Coloboma, microphthalmia, anophthalmia, photoreceptors morphogenesis defects            |                                             |</p>
<table>
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<tr>
<th>Syndrome</th>
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<td>Retinal dystrophy, loss-of-preouter segments</td>
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<td>GLIS2</td>
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<td>Norrie disease, exudative vitreoretinopathy</td>
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<td>LAMA1</td>
<td>150320</td>
<td>Poretti–Boltshauser syndrome</td>
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<td>Senior–Loken syndrome (SLSN)</td>
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<td>TRAF3IP1</td>
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<th>Differential Diagnosis</th>
<th>Zebrafish Model Phenotype</th>
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<tbody>
<tr>
<td>Syndromic optic atrophy</td>
<td>RTN4IP1 610502</td>
<td>Optic atrophy with or without ataxia, mental retardation and seizures</td>
<td>Microphthalmia, absence of retinal ganglion cells and plexiform layers, impaired visual behavior</td>
<td>Angebault et al. (2015)¹</td>
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<td>SLC25A46 610826</td>
<td>Syndromic optic atrophy</td>
<td>Optic nerve atrophy</td>
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<td>Syndromic retinitis pigmentosa</td>
<td>TRNT1 612907</td>
<td>RP and erythrocytic microcytosis, hearing loss</td>
<td>Microphthalmia, impaired visual behavior</td>
<td>Abrams et al. (2015)¹</td>
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<td>Treacher Collins syndrome</td>
<td>TCOF1 606847</td>
<td>Treacher Collins syndrome</td>
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<td>Usher syndrome</td>
<td>MYO7A 276903</td>
<td>USH1B, DFNA11 DFNB2</td>
<td>Prc degeneration, reduced visual function</td>
<td>Ernest et al. (2000)² and Wasfy et al. (2014)²</td>
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<td></td>
<td>USH1C 605242</td>
<td>USH1C, DFNB18</td>
<td>Prc degeneration, reduced visual function</td>
<td>Phillips et al. (2011)¹,² and Blanco-Sanchez et al. (2014)²</td>
</tr>
<tr>
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<td>CDH23 605516</td>
<td>USH1D, DFNB12</td>
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<td>PCDH15 605514</td>
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<td>--------------------------------------------------------------------------------------</td>
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<td>USHRN</td>
<td>USH2A, RP39</td>
<td>Prc degeneration</td>
<td>Ebermann et al. (2010)^1</td>
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<td>ADGRV1</td>
<td>USH2C</td>
<td>Prc degeneration</td>
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<td>USH3A</td>
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<td>PDZD7</td>
<td>Modifier/digenic mutation in USH2 pathology</td>
<td>Prc degeneration</td>
<td>Ebermann et al. (2010)^1</td>
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<td>BCL6</td>
<td>Oculofaciocardiodental and Lenz microphthalmia syndromes;</td>
<td>Microphthalmia; Coloboma</td>
<td>Lee, Lee, and Gross (2013)^1</td>
<td></td>
</tr>
<tr>
<td>BCOR</td>
<td>Oculofaciocardiodental and Lenz microphthalmia syndromes;</td>
<td>Microphthalmia; Coloboma</td>
<td>Ng et al. (2004)^1</td>
<td></td>
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<tr>
<td>EFTUD2</td>
<td>Mandibulofacial dysostosis, Guion-Almeida type with unusual ocular features</td>
<td>Coloboma, microphthalmia</td>
<td>Deml, Reis, Muheisen, Bick, and Semina (2015)^3</td>
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<td>GATA3</td>
<td>Hypoparathyroidism, sensorineural deafness and renal dysplasia</td>
<td>n/a</td>
<td>Sheehan-Rooney, Swartz, Zhao, Liu, and Eberhart (2013)^2</td>
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3. Zebrafish models of syndromes affecting eye and/or ear

Continued
Table 4 Zebrafish Models of Syndromes Affecting Eye and/or Ear—cont’d

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Human Gene/OMIM#</th>
<th>Differential Diagnosis</th>
<th>Zebrafish Model Phenotype</th>
<th>Reference</th>
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<tbody>
<tr>
<td>GJB2 605425</td>
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<td>Bart–Pumphrey syndrome, hystrix-like ichthyosis with deafness, keratitis-ichthyosis-deafness</td>
<td>n/a</td>
<td>Sensorineural hearing loss with variable onset and severity</td>
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<td>PLK4 605031</td>
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<td>Microcephaly, chorioretinopathy</td>
<td>Microphthalmia, reduced retinal growth</td>
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<td>PNPLA6 603197</td>
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<td>Oliver–McFarlane, Laurence–Moon, Boucher–Neuhauser syndromes</td>
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<td>RAB18 602207</td>
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<td>Warburg micro syndrome</td>
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<td>Waardenburg anophthalmia syndrome</td>
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<tr>
<td>Gene</td>
<td>Disease</td>
<td>Ocular Phenotypes</td>
<td>Method of Zebrafish Model Generation</td>
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<td>SOX10</td>
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<td>TUBGCP4</td>
<td>Chorioretinopathy and microcephaly</td>
<td>Microphthalmia, reduced number of pics</td>
<td>Scheidecker et al. (2015)¹</td>
<td></td>
</tr>
</tbody>
</table>

Genes represented in the tables were identified by cross-referencing searches in ZFIN (http://zfin.org), the Online Mendelian Inheritance in Man database (www.omim.org), and PubMed (http://www.ncbi.nlm.nih.gov/pubmed). Criteria for inclusion were restricted to zebrafish models of genes associated with human diseases affecting vision and/or hearing for which ocular or otic phenotypes were described. The following numbers indicate the method used to generate the zebrafish model for a given disease. 1, MO, morpholino; 2, ENU, N-ethyl-N-nitrosourea; 3, TALENs, transcription-activator like effector nucleases; 4, CRISPRs, clustered regularly interspaced short palindromic repeats; 5, Viral insertion. SAG, Statoacoustic ganglion.
the proteins with which harmonin interacts (Adato et al., 2005; Bahloul et al., 2010; Pretorius et al., 2010; Reiners, Marker, Jurgens, Reidel, & Wolfrum, 2005; Reiners, van Wijk, et al., 2005; Siemens et al., 2002; Weil et al., 2003; Wu, Pan, Zhang, & Zhang, 2012). In sensory hair cells, the harmonin scaffold protein binds to MYO7A and CDH23. Harmonin is absolutely required for proper trafficking of these proteins from the ER to the tips of the hair bundle stereocilia and is critical for maintenance of the USH protein complex (Blanco-Sanchez et al., 2014; Boeda et al., 2002; Pan, Yan, Wu, & Zhang, 2009). Failure to maintain this complex results in loss of hair bundle integrity and defective mechanotransduction, and ultimately leads to impaired hearing and balance (Grillet et al., 2009; Michalski et al., 2009).

The role of harmonin in the photoreceptors of the eye is more complex. It is not essential for the function of the USH protein complex in vesicle transport at the periciliary region of the photoreceptors. However, it is critical for synaptic integrity and for the connection between the calycal processes and the outer segments of the photoreceptors (Maerker et al., 2008; Phillips et al., 2011; Sahly et al., 2012). In the intestine, similar to its function in the hair cells, harmonin forms a complex with PCDH24, CDHR2, and MYO7b and is necessary for the correct assembly of the intestinal brush border (Crawley et al., 2014; Li, He, Lu, & Zhang, 2016).

In humans, the spectrum of mutations in USH1C is large, and most mutations lead to syndromic deafness (USH1C) or nonsyndromic autosomal recessive HL (DFNB18) (Ahmed et al., 2002; Bitter-Glindzicz et al., 2000; Bonnet et al., 2016; Ebermann et al., 2007; Ganapathy et al., 2014; Kimberling et al., 2010; Ouyang et al., 2002; Verpy et al., 2000). In addition, mutations in USH1C have been associated with USH2, atypical USH, sector RP with severe HL, and RP with late onset of deafness (Khateb et al., 2012; Le Quesne Stabej et al., 2012; Saihan et al., 2011).

In 5 dpf zebrafish larvae, ush1c is present in the sensory hair cells of the inner ear and the neuromasts, the Müller cells of the retina, and the intestine (Blanco-Sanchez et al., 2014; Phillips et al., 2011). It is also found in adult photoreceptors (Phillips et al., 2011). Like other ush1 zebrafish mutants, vestibular function and hair bundle integrity are disrupted in ush1c depleted larvae (Blanco-Sanchez et al., 2014; Phillips et al., 2011) (Fig. 1). Further investigation of the molecular etiology of these phenotypes in ush1c mutants showed that harmonin is a critical protein necessary for preassembly of Cdh23 and Myo7aa into a complex at the level of the ER, and as described above, mutations in ush1c prevent formation of the complex and its transport to the stereocilia, as well as ER stress, a likely cause of hair cell degeneration (Blanco-Sanchez et al., 2014). Visual function is also affected in ush1c mutants, and maturation of the photoreceptor ribbon synapse is abnormal leading to a decrease in synaptic transmission (Phillips et al., 2011) (Fig. 2). Although the expression of ush1c in the intestine correlates with a possible conserved role of harmonin in the intestinal brush border, its function has not been studied in this tissue.

In summary, the zebrafish ush1c mutant not only recapitulates both the inner ear and retinal phenotypes of USH1 patients, making it an excellent model to study
USH1C, but it also provides insights into the pathways affected in this disease and the mechanism of sensory cell degeneration.

### 3.1.2 USH1B (MYO7A)

MYO7A is another member of the USH family of genes. Mutations in MYO7A, which encodes an unconventional myosin, cause USH1B. In sensory hair cells, MYO7A is involved in hair bundle integrity (Ernest et al., 2000; Inoue & Ikebe, 2003; Nicolson et al., 1998; Udovichenko, Gibbs, & Williams, 2002). Together with other USH1 proteins, MYO7A is thought to anchor the tip links (formed by CDH23 and PCDH15) within the stereocilia (El-Amraoui & Petit, 2014; Richardson et al., 2011). Absence or malfunction of this motor protein results in disorganization of the hair bundle stereocilia (Self et al., 1998). In the eye, MYO7A has been implicated in transport of RPE melanosomes, the transport of opsin in the photoreceptor, and phagocytosis of disk membranes (Gibbs et al., 2004; Gibbs, Kitamoto, Williams, 2003; Liu et al., 1998).

Like USH1C, mutations in MYO7A can give rise to different symptoms based on their nature and position. Mutations typically lead to USHIB, autosomal dominant HL (DFNA11), or autosomal recessive HL (DFNB2) (Bolz et al., 2004; Di Leva et al., 2006; Duman, Sirmaci, Cengiz, Ozdag, & Tekin, 2011; Kimberling et al., 2010; Liu, Newton, Steel, & Brown, 1997; Liu, Walsh, et al., 1997; Mutai et al., 2013; Riazuddin et al., 2008; Roux et al., 2011; Street, Kallman, & Kiemele,...
In other cases, however, mutations in MYO7A have been associated with USH2 or atypical USH (Bonnet et al., 2011; Le Quesne Stabej et al., 2012; Roberts, George, Greenberg, & Ramesar, 2015; Rong et al., 2014; Roux et al., 2011; Zhai et al., 2015).

Zebrafish have two MYO7A genes, myo7aa and myo7ab. myo7aa is better characterized. It is expressed in the sensory hair cells of the inner ear as early as 24 hpf, when the first hair cells start to differentiate, and expression persists throughout their

FIGURE 2 Maturation of the photoreceptors ribbon synapses.

Transmission electron micrographs of cone pedicles at 6 dpf in wild-type (A,C) and ush1c morphant (B,D) larvae. Multiple triads (boxed areas in A and B and enlargements in C and D) consisting of a synaptic ribbon (r), arciform density (arrow) and postsynaptic processes from bipolar (b) and horizontal (h) cells indicate synaptic maturation. Scale bars 1 µm (A,B) and 500 nm (C,D). Electroretinogram recordings at 5 dpf showing b-waves (E) and a-wave (F) amplitudes from wild-type (uninjected control) and ush1c morphant larvae.

development (Ernest et al., 2000). It is also detected in the neuromasts from 48 hpf (Ernest et al., 2000). Myo7aa is localized in both the cytoplasm and along the stereocilia of the hair bundle (Blanco-Sanchez et al., 2014; Coffin, Dabdoub, Kelley, & Popper, 2007). In the eye, myo7aa is expressed in the inner and outer nuclear layers (Wasfy, Matsui, Miller, Dowling, & Perkins, 2014). The protein is also found in the accessory outer segment of cone photoreceptors (Hodel et al., 2014).

Multiple alleles of myo7aa (mariner) mutants have been analyzed and all display vestibular dysfunction (Nicolson et al., 1998). The splayed stereocilia of the hair bundles and the resulting defective mechanotransduction are responsible for this phenotype (Ernest et al., 2000; Nicolson et al., 1998; Seiler & Nicolson, 1999) (Fig. 1). A recent study described the retinal phenotypes in myo7aa mutants that include diminished visual function and degeneration of rod photoreceptors (Wasfy et al., 2014). As observed in Myo7a mouse mutants, opsins are not correctly localized; rhodopsin accumulates near the connecting cilium and blue cone opsin partially mislocalizes in the inner segment.

### 3.1.3 USH1D (CDH23) and USH1F (PCDH15)

CDH23 and PCDH15 encode transmembrane proteins known for their role in maintaining the integrity of the inner ear hair cell mechanoreceptors (Boeda et al., 2002; Di Palma et al., 2001; Kikkawa, Pawlowski, Wright, & Alagramam, 2008; Seiler et al., 2005; Söllner et al., 2004). Specifically, CDH23 together with PCDH15 forms the tip links that connect stereocilia together (Kazmierczak et al., 2007). Preservation of these links is critical for mechanosensation, and any disruption leads to defective hearing and balance (El-Amraoui & Petit, 2005).

As with other USH genes, mutations in CDH23 can give rise to syndromic or nonsyndromic deafness. In most cases, splice site, frameshift, and nonsense mutations give rise to USHID, whereas missense mutations are more likely to generate autosomal recessive HL (DFNB12) (Ammar-Khodja et al., 2009; Astuto et al., 2002; Bork et al., 2001). Interestingly, a few patients with atypical USH (milder symptoms in either hearing, balance, or vision), USH2, or USH3 have also been reported with mutations in CDH23 (Astuto et al., 2002; Besnard et al., 2014; Bonnet et al., 2011; Le Quesne Stabej et al., 2012). Similarly, mutations in PCDH15 can lead to USH1F or autosomal recessive HL (DFNB23), with missense mutations tending to cause DFNB23 (Ahmed et al., 2003, 2008; Doucette et al., 2009).

In zebrafish, a panoply of mutants for cdh23 has been generated and studied. Based on several behavioral assays (Table 1), the cdh23 (sputnik) mutants were characterized with auditory and vestibular defects (Nicolson et al., 1998). Further studies of the mutants, focusing on the morphology of the mechanoreceptors in hair cells of the crista, showed that the stereocilia are detached from the kinocilium and splayed (Nicolson et al., 1998; Söllner et al., 2004) (Fig. 1). Micropotentials that measure the extracellular voltage response are absent in the mutants, and the FM1-43 dye, a marker of cycling vesicles, does not incorporate into the hair cells by endocytosis (Nicolson et al., 1998; Seiler & Nicolson, 1999), consistent with a disruption in mechanotransduction.
Although all the data point toward zebrafish cdh23 mutants being excellent models to study mechanotransduction defects, several observations suggest they may not be appropriate for studies of combined deafness and blindness. First, using in situ hybridization techniques, cdh23 expression was found in a subset of GABAergic amacrine cells, but not in photoreceptors of larval zebrafish eyes (Glover et al., 2012). Second, optokinetic reflex measurements (OKR) showed normal visual function for two alleles of cdh23 (Glover et al., 2012; Mo, Chen, Nechiporuk, & Nicolson, 2010). One of the alleles, however, was a missense mutation, and as discussed above, missense mutations in humans typically lead to HL only whereas nonsense or frameshift mutations generate USH1D. It is therefore possible that the alleles studied are not adequate models of USH. In addition, the behavioral analysis in this study was conducted at 5 dpf. At the same stage, myo7aa mutants do not yet show retinal cell death, and visual function assayed by electroretinogram and the OKR is only diminished (Wasfy et al., 2014). Experiments at later time points could help determine whether cdh23 mutants can be used as models for USH.

Zebrafish have two orthologs of human PCDH15, pcdh15a and pcdh15b. pcdh15a is expressed in the eye, mechanosensory hair cells, and brain, whereas the pcdh15b paralog is expressed in photoreceptors, the brain, and weakly in the inner ear and neuromasts (Seiler et al., 2005).

Alleles of pcdh15a (orbiter) were isolated in the same screen for vestibular dysfunction as the cdh23 (sputnik) mutants (Nicolson et al., 1998). Similar to other ush1 mutants, pcdh15a depleted larvae display splayed stereocilia at 5 dpf and disrupted mechanotransduction (Nicolson et al., 1998; Seiler et al., 2005). At 4 dpf, the OKR is normal. Interestingly, the complementary phenotype is observed in pcdh15b morphant larvae. Vestibular function and mechanotransduction are unaffected, but visual function is impaired (Seiler et al., 2005). With the lack of an OKR defect, pcdh15a mutants do not seem to recapitulate the vision defects of USH1F patients. However, like cdh23 mutants, visual function was assayed early in pcdh15a mutants, leaving open the question of whether they provide a good model for USH1F.

### 3.1.4 Potential models of USH
#### 3.1.4.1 Usher syndrome type 2
Zebrafish mutants and morphants of USH2 (ush2a and adgrv1) and USH3 (clrn1) genes have also been generated. Although comprehensive studies are still incomplete (i.e., only visual or vestibulo-acoustic function has been reported), the results are promising and suggest these mutants may potentially be useful models of USH.

Zebrafish injected with MOs against ush2a or adgrv1 (USH2C) (Ebermann et al., 2010) exhibit retinal degeneration in the first week of life. No vestibular defects were described. Depleting either of these genes in conjunction with knockdown of the scaffold protein-encoding gene pdzd7 exacerbates the retinal symptoms, providing supporting evidence for clinical findings implicating PDZD7 as a modifier of USH2. USH2 is unique among the three subtypes for its lack of reported vestibular dysfunction, so there is reasonable hope that zebrafish USH2 models may be spared...
the lethal swimming and balance problems characteristic of the USH1 and USH3 models described here. This could allow for long-term assays of visual function and retinal cell loss as well as analysis of auditory function uncoupled from the vestibular system.

3.1.4.2 Usher syndrome type 3
USH3A is caused by mutations in the tetraspanin protein-encoding gene $CLRN1$. Investigations of zebrafish morphants and mutants (Gopal et al., 2015; Ogun & Zallocchi, 2014) have demonstrated the conserved role of $clrn1$ in hearing and balance, with both models recapitulating the progressive onset of symptoms. Retinal assays were not described by either group, but $Clrn1$ is present in larval and adult eyes (Phillips et al., 2013), suggesting a potential for a retinal phenotype in loss-of-function models. The late onset of hearing and balance defects in USH3 patients presents a novel window for preventative therapies, and the zebrafish $clrn1$ model could serve as an important component of drug discovery.

3.2 JOUBERT SYNDROME
Zebrafish were used in a candidate gene study and clinical validation of the centrosomal protein-encoding gene $POC1B$ as causative of Joubert syndrome (JBTS) and the nonsyndromic retinal ciliopathy Leber congenital amaurosis. Two studies of JBTS patients with loss-of-function mutations in $POC1B$ (Beck et al., 2014; Roosing et al., 2014) used zebrafish to validate genotype—phenotype correlation in ciliopathy patients. Another MO-based study demonstrated retinal defects by $poc1b$ depletion in zebrafish (Zhang, Zhang, Wang, & Liu, 2015).

Interestingly, although the clinical symptoms of JBTS and other ciliopathies manifest in a broad range of ciliated cell populations, SNHL is infrequently reported (reviewed in Waters & Beales, 2011). In contrast, there are numerous examples of ciliopathy gene defects affecting zebrafish ear morphology, often including defective otolith development (Beck et al., 2014; Kim et al., 2013; Lee et al., 2012; Omori et al., 2008; Simms et al., 2012; see Table 4). The reason for this discrepancy is unknown, but ciliopathies encompass a range of severe neurological and systemic defects, which may make hearing or balance difficulties more challenging to diagnose. In support of this, a recent study of patients with primary ciliary dyskinesia that was motivated by the otolith defects observed in zebrafish ciliopathy models revealed that PCD patients have measurable vestibular defects (Rimmer et al., 2015).

CONCLUSION
Mutagenesis screens have generated a broad array of loss-of-function models that shed light on numerous mechanisms of eye and ear development and disease. Despite the numerous disease models presented in this chapter, there is still a
significant discrepancy between the number of distinct genetic diseases and the availability of tractable mutant or MO models. To date, 250 genes have been associated with vision loss in humans (OMIM, 2016), but only 25 mutant models and 64 MO zebrafish models targeting 79 genes have been studied for vision defects (Tables 2 and 4). Similarly, 115 genes are linked to deafness in humans (OMIM, 2016), but only 22 mutant and 24 MO zebrafish models targeting 40 genes have been studied for hearing defects (Tables 3 and 4). In addition, the rate of discovery of disease-causing genes is accelerating due to advanced sequencing methods in use clinically. Continued efforts of the zebrafish research community are vital not only for gene discovery, but also to increase our understanding of the cellular and molecular processes involved in vision and hearing. The Sanger zebrafish mutation project and the new technologies of genome editing provide essential resources for generating new models (Ata, Clark, & Ekker, 2016; Busch-Nentwich et al., 2013; Kettleborough et al., 2013). Additional resources, such as high-throughput methods for efficient assays of vision and hearing impairment and new tools to study the molecular pathology in mutants, will also be helpful. The utility of behavioral assays for auditory and vestibular dysfunction was demonstrated in forward and reverse screens and mutant analyses (Abbas & Whitfield, 2009; Han et al., 2011; Kindt, Finch, & Nicolson, 2012; Malicki et al., 1996; Nicolson et al., 1998; Varshney et al., 2015; Whitfield et al., 1996; Yao, DeSmidt, Tekin, Liu, & Lu, 2016).

Collectively, increased use and optimization of gene editing techniques in the zebrafish research community, alongside increased genotypic data emerging from human patients and more collaborations between clinicians and model organism researchers, will produce new models of vision and hearing disorders that will lead to better diagnoses and treatments of human disease.

REFERENCES


role in brain, retinal, and renal development. *Cellular and Molecular Life Sciences, 69*, 993–1009.


