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A mouse model for benign paroxysmal positional vertigo (BPPV) with genetic predisposition for displaced otoconia

Amiel A. Dror[1,2,3], **Shahar Taiber**[1], **Eyal Sela**[2,3], **Ophir Hendzel**[4,5], **Karen B. Avraham**[1]

[1]Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

[2]Department of Otolaryngology-Head and Neck Surgery, Galilee Medical Center, Nahariya, Israel

[3]Faculty of Medicine in the Galilee, Bar-Ilan University, Safed, Israel

[4]Department of Otolaryngology Head and Neck Surgery and Maxillofacial Surgery, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel

[5]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Abstract

Abnormal formation of otoconia, the biominerals of the inner ear, results in balance disorders. The inertial mass of otoconia activates the underlying mechanosensory hair cells in response to change in head position primarily during linear and rotational acceleration. Otoconia associate exclusively with the two gravity receptors, the utricle and saccule. The cristae sensory epithelium is associated with an extracellular gelatinous matrix known as cupula, equivalent to otoconia. During head rotation, the inertia of endolymphatic fluids within the semicircular canals deflect the cupula of the corresponding crista and activates the underlying mechanosensory hair cells. It is believed that detached free floating otoconia particles travel ectopically to the semicircular canal and cristae and are the culprit for benign paroxysmal positional vertigo (BPPV). The *Slc26a4* mouse mutant harbors a missense mutation in pendrin. This mutation leads to impaired transport activity of pendrin and to defects in otoconia composition and distribution. All $Slc26a4^{loop/loop}$ homozygous mutant mice are profoundly deaf but show inconsistent vestibular deficiency. A panel of behavioral tests was utilized in order to generate a scoring method for vestibular function. A pathological finding of displaced otoconia was identified consistently in the inner ears of mutant mice with severe vestibular dysfunction. In this work, we present a mouse model with a genetic predisposition for ectopic otoconia with a clinical correlation to BPPV. This unique mouse model can serve as a platform for further investigation of BPPV pathophysiology, and for developing novel treatment approaches in a live animal model.

Correspondence Karen B. Avraham, Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel., karena@tauex.tau.ac.il.

CONFLICTS OF INTEREST

The authors declare no conflict of interests in the current study.

DATA AVAILABILITY STATEMENT

Data is available in Supporting information.

Keywords

SLC26A4; balance; vertigo; hearing; deafness

INTRODUCTION

Among peripheral vestibular disorders, Benign Paroxysmal Positional Vertigo (BPPV) is the most common clinical condition.[1] In 1952, Dix and Hallpike proposed a pathophysiological mechanism for BPPV, presuming that ectopic otoconia debris in the vestibular labyrinth triggers a pathological sensation of vertigo.[2] This simple bedside test, whereby laying a patient down into a supine position provokes a 'spinning' feeling, with rotatory eye nystagmus lasting for a short duration.

The hypothesis of ectopic crystal as the culprit of BPPV is supported by a successful treatment approach, aimed to reposition these ectopic crystals to their native anatomical position. The Epley maneuver,[3] also known as the canalith repositioning procedure (CRP), is named after Dr. John Epley, who developed a sequential stepwise treatment of rapid changes in body position with a short interval between each step. The Epley procedure, among other CRPs such as Semont,[4] Foster, and Brandt-Daroff[5] maneuvers, are highly beneficial in treating BPPV.[6] Two randomized controlled studies reported that CRP has more than a 90% success rate treating BPPV as compared to placebo control.[7, 8]

While the majority of BPPV events are idiopathic in nature, the most common cause for post traumatic BPPV is head trauma.[9] The abrupt high-velocity energy potentially displaces otoconia particles that trigger BPPV events. Additional support for ectopic otoconia in BPPV etiology comes from the increase of newly diagnosed vestibular dysfunction among astronauts during space travel and upon returning to earth.[10, 11] In the absence of gravity force, it is proposed that weightless otoconia particles dissociate from the extracellular matrix, and evoke pathological vestibular sensation. Age-related morphological changes in otoconia maintenance also lead to degeneration of particles that increase the likelihood of floating extracellular debris in the endolymph.[12] This observation may explain the higher incidence of BPPV among elderly patients.[13] With the limitation of currently available imaging modalities in identifying tiny otoconia crystals, the histological analysis of cadaveric temporal bone provides a retrospective study of patients who suffered from vestibular dysfunction. Despite the self-limiting nature of BPPV, surgical treatment is reserved for incapacitating BPPV. The first *in-vivo* evidence for "free-floating-particles" was identified during a canal occlusion procedure aimed to abolish endolymphatic flow in the posterior semicircular canal.[14] Additional studies consistently found a particulate matter composed of otolithic membrane and otoconia in the semicircular canals of patients with BPPV.[15, 16]

Studies on animal models such as mice and zebrafish have provided invaluable information on otoconia development, composition and morphology.[17] This uniquely regulated process of extracellular assembly of mineralized bodies depends on temporal and spatial expression of more than 70 protein-coding genes. We have previously reported that *Slc26a4*$^{loop/loop}$ harbors a missense in pendrin, a member of the SLC family of ion transporters.[18] This

mutation leads to impaired transport activity of pendrin and to defects in otoconia composition and distribution. Despite the identical inbred genetic background of mutants, their vestibular dysfunction is highly variable. While some mutant mice show remarkable normal vestibular function, others show severe dysfunction in vestibular behavioral testing. The data collected in the following study show that the presence of ectopic otoconia provokes severe vestibular dysfunction in mice. The proposed mechanism of ectopic mineralized bodies as the culprit of vestibular dysfunction is supported by this first genetic mouse model for BPPV.

MATERIAL AND METHODS

Mice

All procedures involving animals were approved and met the guidelines described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee (IACUC) of Tel Aviv University (01–16-100). All mice were group housed and tested during the light phase.

Genotyping

A genotyping assay was performed to distinguish between *Slc26a4*$^{loop/loop}$ mutant and wild-type mice, both on a C3HeB/FeJ genetic background, as previously described.[18]

Behavioral testing

Auditory brainstem response (ABR)was evaluated as previously described[18]. To evaluate vestibular function of mutant mice, we utilized a battery of behavioral tests with standardized scoring systems. For the swimming test, the mouse was gently inserted into a bath of warm water and watched carefully. Normal swimming performance were scored with zero, demonstrating regular performance. Mice with irregular swimming were scored with one point, while mice with immobile floating were scored with two points. Mice with an inability to swim were rescued immediately to avoid drowning and ranked with the maximum score of three points. For the trunk curl test, the mouse was gently hung from his tail 10 cm above a clean surface with a metal grid. Normal behavior was demonstrated by a reaching position, with a score of zero, by the extension of limb and head forward and downward aiming to the floor. Mice with an abnormal behavior, ranked with a score of 1, tried to climb towards the examiner's hand, curling the body upward reaching with its head to the tail. The remaining three behavioral tests were based on observation of each mice in a clean cage. A positive circling behavior was scored with 1 point, while a head tilt gained another point. For gait observation, we defined a scale of zero (normal gait) to three points (incapacity) for mice unable to walk with a typical habitus of a tilted trunk from head to tail lying on the cage floor. All mice in this study were tested between 8–10 weeks of age. The score of each test was normalized and a total sum of the cumulative score was calculated upon completion of the behavioral pipeline, giving each test 20 percent of the final score.

Visualization of the endolymphatic labyrinth

Inner ears were gently isolated and fixation was done in 4% paraformaldehyde (Electron Microscopy Sciences) in Dulbecco's phosphate-buffered saline (D-PBS) overnight at 4 °C.

After a single wash in D-PBS, further fine dissections were conducted under the binocular (Leica) for removing the bony envelop surrounding the endolymphatic labyrinth of the vestibule. Grip forceps were used to gently fix the cochlear portion leaving the vestibular part exposed for further dissection. For the next step we utilized a scalpel handle equipped with replaceable blade No. 11, to gently shave the bony envelope of vestibular endolymphatic labyrinth with careful attention not to compromise the associated membranous portion. For each inner ear dissection, at least two blades were replaced to maintain a sharp edge that reduce the compression needed on the tissue and avoids bone fractures. Counting of the mineralized body structure in the utricle, saccule and in ectopic position either canalithiasis (within the semicircular canal) or cupulolithiasis (within the cristae and associated cupula) were documented for each ear in a systematic manner.

Statistical analysis

Data are presented as means ± SD unless noted otherwise in the results section. Sample sizes are noted on the figures. Statistical analysis was performed using Prism 8 (GraphPad, San Diego, CA, USA). Data were tested for normality using the D'Agostino and Pearson, Shapiro-Wilk and Kolmogorov-Smirnov tests, and whenever multiple comparisons were performed, Dunn, Tukey, or Bonferroni were used to adjust P values.

RESULTS

Slc26a4$^{loop/loop}$ mutant mice are consistently deaf but show variable incidence of ectopic otoconia in vestibular system

All *Slc26a4*$^{loop/loop}$ mice were profoundly deaf according to auditory brainstem response (ABR) testing (Figure 1). Tone-burst ABR tests were performed on 6–12 weeks old *Slc26a4*$^{loop/loop}$ mice with (+) (n=5) or without (−) (n=6) circling behavior, and wild-type mice (n=14). Data are mean ± SD. Statistical test by two-way ANOVA with post-hoc Tukey correction for multiple comparisons: ****P<0.0001. Arrows indicate no observable response. To evaluate the vestibular function of mutant mice, we utilized a battery of behavioral tests with standardized scoring systems. Following the behavioral test pipeline, the inner ears of all mutant mice were isolated and fixed for pathological characterization. Fine dissection was performed on both ears of each mouse with gentle exposure of the endolymphatic labyrinth. Anatomical mapping of the giant otoconia was documented (Figure 2a–e). As expected from our previous study, *Slc26a4*$^{loop/loop}$ mutant mice had a significantly higher tendency to have giant ectopic otoconia in their membranous labyrinth compared to wild type mice (41.8% versus 0% respectively, P=0.0009, two-sided Fisher's exact test (Figure 2f). The observed ectopic mineralized bodies were identified either in the cupula (cupolithiasis) or the associated semicircular canals (canalolithiasis) (Figure 2c, Figure 4).

Displaced otoconia in vestibular labyrinth is associated with severe vestibular dysfunction in *Slc26a4*$^{loop/loop}$ mutant mice

The principal of each behavioral test is illustrated (Figure 3a–d) and a comprehensive scoring system is listed (Figure 3e). All mice in this study were tested between 8–10 weeks of age. A total sum of the cumulative score was calculated upon completion of the

behavioral pipeline (Figure 2f), giving each test an equal weight of 20 percent. A group of wild-type mice (16 mice, 8 males and 8 females) were used as controls for the behavioral testing. A prominent variable vestibular phenotype among mutant mice was observed. Each mouse was tested using all behavioral assays and was ranked according to the severity scale associated with each test (Figure 2e, f). We found sex to have no effect on the vestibular score of the mice (P= 0.9278, Mann-Whitney; Figure S1, Supporting information). The proportion of mice presenting a vestibular phenotype in each vestibular behavioral test was consistently significantly higher in the ectopic otoconia group compared to loop mice with no ectopic otoconia (Figure 2f). Statistical test by Fisher's exact test with Bonferroni correction for multiple comparisons: ****P<0.0001, ***P<0.001, *P<0.05. Comparison of the cumulative vestibular score of the two groups showed a significant difference as well (Figure 2g). Data are median ± 95% CI of the cumulative score. Statistical test by Kruskal-Wallis test with post-hoc Dunn's correction for multiple comparisons: **P<0.01, ****P<0.0001. Males and females had ectopic otoconia at similar rates (38.46% of males and 45% of females, P=0.556, two-sided Chi-square, supplementary 2a). In addition, the ectopic otoconia were found at both ears at similar rates (21.52% of right ears and 22.78% of left ears, P=0.848, two-sided Chi-square; Figure S2, Supporting information).

DISCUSSION

Dizziness is a very common complaint in a patient seeking medical attention, and yet it remains a complex diagnostic challenge. Unsteady gait, for example, can be the first alarming presentation of a cerebrovascular event, while on the other hand, it could be a benign presentation of peripheral vertigo. The clinician, therefore, requires a high index of suspicion, careful anamnestic attention and clinical examination to avoid the delayed or misdiagnosis of a fatal condition. Years before the availability of advanced vestibular testing, Dix and Hallpike proposed a simple, yet accurate, diagnostic method for differentiating between peripheral and central cause of dizziness.[2] The Dix and Hallpike test remains a prominent integral part of a physical examination during bedside evaluation of a 'dizzy' patient. The successful treatment approach with reposition maneuvers for patient diagnosed with BPPV highlight the valuable diagnostic power of Dix and Hallpike physical exam. The etiology of BPPV, however, is not fully understood, at least for some of the affected individuals. Whereas different factors presume to contribute for BPPV, such as head trauma, in many cases the specific initiating event remains unknown.

Here we show that a genetic mutation in the *Slc26a4* gene leads to profound deafness in all mutant *Slc26a4*$^{loop/loop}$ mice. Despite an identical inbred genetic background, a subgroup of *Slc26a4*$^{loop/loop}$ mutant mice shows severe vestibular dysfunction, while the rest of the mutant mice show exceptionally normal vestibular function, undifferentiated from wild-type mice. The inconsistent vestibular phenotype among *Slc26a4*$^{loop/loop}$ mice is unlikely due to variable penetrance of the *Slc26a4 loop* mutation, given the consistent deafness phenotype in all mutants. Moreover, the variable vestibular behavior is reported in an additional mouse model for Slc26a4.[19] Only 29% of Slc26a4 Leu236Phe knock-in mouse display severe vestibular dysfunction, while the majority of mutants performed exceptionally normal on the rotarod test, undifferentiated from wild type. The *Slc26a4*$^{tm1Dontuh/tm1Dontuh}$ mice reported

to have head-tilting and circling behavior in approximately 46%[20], while the *Pds*$^{-/-}$ mice show vestibular dysfunction in about 50% of the cases.[21]

Prior to a comprehensive behavioral examination, a quick observation of a cage with a group of *Slc26a4*$^{loop/loop}$ mutant mice is sufficient to differentiate between mutants with and without a vestibular deficiency. The prominent unsteady gait, rapid circling behavior, or alternatively tilted body with decreased motor activity is a prominent hallmark of the subgroup of *Slc26a4*$^{loop/loop}$ mutants with vestibular deficiency. In a previous study, we described the defect in otoconia nucleation and composition of *Slc26a4*$^{loop/loop}$ mice[18]. The existence of an abnormal mineralized body in the utricle and saccule of *Slc26a4* mutant mice already at birth suggests that developmental defects in otoconia occur with impaired pendrin function. Seeding of otoconia begins early in development and require a series of sequential events, both temporally and spatially, to support this extracellular process.[17, 22] Every single otoconium with an average size of 2–5 um is composed of an organic core encased in a mineralized rhomboid-shaped envelope. The first step in otoconia seeding requires the secretion of core glycoproteins and proteoglycans to the immediate extracellular endolymphatic microenvironment at the apical surface of utricular and saccular maculae.[23] The protein conglomerate of the otoconium seed is rich with negatively-charged amino acid residues that serve as a scaffold for deposition of the Ca^{2+} to form the calcite mineralized otoconia. As expected, a genetic mutation in the major otoconia matrix protein of mammals, Otoconin-90 (Oc90), results in failure of otoconia seeding and abnormal otoconia.[24] The presence of an otoconia formation defect, however, is not limited to mutations of otoconium core proteins, but rather influenced by a long list of protein-coding genes.[17] Dysfunction of proton pumps and transporters alter endolymph homeostasis and leads to failure of otoconia formation. Lack of pendrin transport activity leads to abolishment of HCO_3^- excretion and acidification of endolymphatic fluids.[25] As a consequence, inhibition of pH-sensitive TRPV5 and TRPV6 channels leads to pathological elevation of endolymphatic Ca^{2+} concentration.[26] Endolymph homeostasis is crucial during inner ear development but is also essential for the maintenance of otoconia through adulthood. We previously proposed that luminal acidification impairs normal turnover of otoconia mineralized fraction progressively in adult *Slc26a4*$^{loop/loop}$ mutants.[18] The abnormal calcite mineralized body observed in newborn mice is eventually replaced with calcium oxalate crystal in adults. We presume that a pH-dependent dissolution of endolymphatic calcite otoconia, through intermediate phasic formation of weddellite crystals, leads to precipitation of stable calcium oxalate that favor acidic environment. Interestingly, despite the dramatic changes in otoconia size and mineralized content in *Slc26a4*$^{loop/loop}$, our data suggest that abnormal formation and composition of giant otoconia by itself is not necessarily sufficient to provoke severe vestibular dysfunction. This observation is supported by different mouse models with absent or abnormal otoconia that performed normally in terms of vestibular function, such as in a swimming test.[27, 28] Recording of linear vestibular evoked potentials (VsEPs) were obtained in mice with abnormal otoconia, an indication of preserved vestibular function. The intact vestibular sensory hair cells of *Slc26a4* mutant mice preserve a normal morphology and structure and can potentially maintain efferent vestibular stimuli.[18, 19] The group of *Slc26a4*$^{loop/loop}$ mutant with normal vestibular function indicates that afferent vestibular pathway through Scarpa's ganglion to vestibular cortex is functional. Our findings suggest

that the existence of ectopic mineral is the culprit in the subgroup of *Slc26a4*$^{loop/loop}$ mutant mice with severe vestibular dysfunction. Fine dissection of vestibule of mutant mice with normal vestibular function reveals giant otoconia over the saccule and utricle. However, only subgroup of mutant mice *Slc26a4*$^{loop/loop}$ appears to have ectopic otoconia, with striking correlation to a severe vestibular dysfunction. The presence of ectopic otoconia in this subgroup of mutants with vestibular dysfunction can be explained by two possible mechanisms. The most reasonable hypothesis suggests that an ectopic giant stone was originally formed over the maculae and at some point, changed its position ectopically. The enlarged vestibular aqueduct (EVA) observed in *Slc26a4*$^{loop/loop}$ increases the chances of spontaneous reposition of giant otoconia through a significantly larger opening of the vestibular labyrinth. Another possible mechanism is the formation of the giant stone ectopically. Such a mechanism is less likely to occur frequently, but as we have shown in previous studies,[18] a dynamic pH-dependent mechanism leads to dissolution and precipitation of newly-formed mineralized bodies in distant anatomic positions (Figure 4).

In summary, in this study, we show for the first time that a genetic mutation predisposes to BPPV in a mouse model. Although the majority of BPPV patients do not carry a *SLC26A4* mutation, our study highlights that a given genetic background can potentially predispose an individual to develop BPPV in different circumstances. A genetic mutation in otoconial adhesion or a structural protein may not be sufficient to lead to BPPV by itself, but during head trauma, an affected individual may have a greater risk for displaced otoconia and vertigo. The existence of a mouse model for BPPV serves as a valuable platform to further investigate environmental factors or physical activity that potentially provoke or alleviate BPPV. Further studies of the possible influence of comorbidities such as endocrinopathy and osteoporosis can highlight the importance of medical treatments of other diseases to improve BPPV symptoms. Such important knowledge can provide individuals with a known familial tendency for BPPV to avoid certain habits or clinical conditions bearing a greater risk for provoking events. Identifying additional genetic factors that predispose to BPPV will contribute to an in-depth understanding of the molecular pathophysiology of BPPV, and the potential development of additional medical treatments for patient with persistent BPPV through a pharmacogenetics approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Nedzelski JM, Barber HO, McIlmoyl L. Diagnoses in a dizziness unit. J Otolaryngol. 1986;15:101–104. [PubMed: 3712537]
2. Dix MR, Hallpike CS. The pathology, symptomatology and diagnosis of certain common disorders of the vestibular system. Proc R Soc Med. 1952;45:341–354. [PubMed: 14941845]

3. Epley JM. New dimensions of benign paroxysmal positional vertigo. Otolaryngol Head Neck Surg. 1980;88:599–605. [PubMed: 7443266]
4. Semont A, Freyss G, Vitte E. Curing the BPPV with a Liberatory Maneuver In: Clinical Testing of the Vestibular System. Karger Publishers; 1988 p. 290–3.
5. Brandt T, Daroff RB. Physical therapy for benign paroxysmal positional vertigo. Arch Otolaryngol - Head Neck Surg. 1980;106:484–485.
6. Parnes LS, Agrawal SK, Atlas J. Diagnosis and management of benign paroxysmal positional vertigo (BPPV). CMAJ. 2003;169:681–693. [PubMed: 14517129]
7. Sacco RR, Burmeister DB, Rupp VA, Greenberg MR. Management of benign paroxysmal positional vertigo: a randomized controlled trial. J Emerg Med. 2014;46:575–581. [PubMed: 24462034]
8. Froehling DA, Bowen JM, Mohr DN, Brey RH, Beatty CW, Wollan PC, Silverstein MD. The canalith repositioning procedure for the treatment of benign paroxysmal positional vertigo: a randomized controlled trial. Mayo Clin Proc. 2000;75:695–700. [PubMed: 10907384]
9. Baloh RW, Honrubia V, Jacobson K. Benign positional vertigo: clinical and oculographic features in 240 cases. Neurology. 1987;37:371–378. [PubMed: 3822129]
10. Shelhamer M Trends in sensorimotor research and countermeasures for exploration-class space flights. Front Syst Neurosci. 2015;9:115. [PubMed: 26321927]
11. Gurovskiy NN, Bryanov II, Yegorov AD. Changes in the vestibular function during space flight. Acta Astronaut. 1975;2:207–216. [PubMed: 11887913]
12. Jang YS, Hwang CH, Shin JY, Bae WY, Kim LS. Age-related changes on the morphology of the otoconia. Laryngoscope. 2006;116:996–1001. [PubMed: 16735917]
13. Babin RW, Harker LA. The vestibular system in the elderly. Otolaryngol Clin North Am. 1982;15:387–393. [PubMed: 6979732]
14. Parnes LS, McClure JA. Free-floating endolymph particles: a new operative finding during posterior semicircular canal occlusion. Laryngoscope. 1992;102:988–992. [PubMed: 1518363]
15. Kao WTK, Parnes LS, Chole RA. Otoconia and otolithic membrane fragments within the posterior semicircular canal in benign paroxysmal positional vertigo. Laryngoscope. 2017;127:709–714. [PubMed: 27726156]
16. Welling DB, Parnes LS, O'Brien B, Bakaletz LO, Brackmann DE, Hinojosa R. Particulate matter in the posterior semicircular canal. Laryngoscope. 1997;107:90–94. [PubMed: 9001271]
17. Lundberg YW, Xu Y, Thiessen KD, Kramer KL. Mechanisms of otoconia and otolith development. Dev Dyn. 2015;244:239–253. [PubMed: 25255879]
18. Dror AA, Politi Y, Shahin H, Lenz DR, Dossena S, Nofziger C, Fuchs H, Hrabé de Angelis M, Paulmichl M, Weiner S, Avraham KB. Calcium oxalate stone formation in the inner ear as a result of an *slc26a4* mutation. J Biol Chem. 2010;285:21724–21735. [PubMed: 20442411]
19. Wen Z, Zhu H, Li Z, Zhang S, Zhang A, Zhang T, Fu X, Sun D, Zhang J, Gao J. A knock-in mouse model of Pendred syndrome with Slc26a4 L236P mutation. Biochem Biophys Res Commun. 2019;515:359–365. [PubMed: 31155292]
20. Lu Y-C, Wu C-C, Shen W-S, Yang T-H, Yeh T-H, Chen P-J, Yu I-S, Lin S-W, Wong J-M, Chang Q, Lin X, Hsu C-J. Establishment of a knock-in mouse model with the SLC26A4 c.919–2A>G mutation and characterization of its pathology. PLoS One. 2011;6:e22150. [PubMed: 21811566]
21. Everett LA, Belyantseva IA, Noben-Trauth K, Cantos R, Chen A, Thakkar SI, Hoogstraten-Miller SL, Kachar B, Wu DK, Green ED. Targeted disruption of mouse *Pds* provides insight about the inner-ear defects encountered in Pendred syndrome. Hum Mol Genet. 2001;10:153–161. [PubMed: 11152663]
22. Hughes I, Thalmann I, Thalmann R, Ornitz DM. Mixing model systems: Using zebrafish and mouse inner ear mutants and other organ systems to unravel the mystery of otoconial development. Brain Res. 2006;1091:58–74. [PubMed: 16529728]
23. Ross MD, Peacor DR. The nature and crystal growth of otoconia in the rat. Ann Otol Rhinol Laryngol. 1975;84:22–36.
24. Zhao X, Yang H, Yamoah EN, Lundberg YW. Gene targeting reveals the role of Oc90 as the essential organizer of the otoconial organic matrix. Dev Biol. 2007;304:508–524. [PubMed: 17300776]

25. Wangemann P, Nakaya K, Wu T, Maganti RJ, Itza EM, Sanneman JD, Harbidge DG, Billings S, Marcus DC. Loss of cochlear HCO3- secretion causes deafness via endolymphatic acidification and inhibition of Ca^{2+} reabsorption in a Pendred syndrome mouse model. Am J Physiol Renal Physiol. 2007;292:1345–1353.

26. Nakaya K, Harbidge DG, Wangemann P, Schultz BD, Green ED, Wall SM, Marcus DC. Lack of pendrin HCO3- transport elevates vestibular endolymphatic [Ca2+] by inhibition of acid-sensitive TRPV5 and TRPV6 channels. AJP Ren Physiol. 2007;292:1314–1321.

27. Jones SM, Erway LC, Johnson KR, Yu H, Jones TA. Gravity receptor function in mice with graded otoconial deficiencies. Hear Res. 2004;191:34–40. [PubMed: 15109702]

28. Swank RT, Reddington M, Howlett O, Novak EK. Platelet storage pool deficiency associated with inherited abnormalities of the inner ear in the mouse pigment mutants muted and mocha. Blood. 1991;78:2036–44. [PubMed: 1912584]

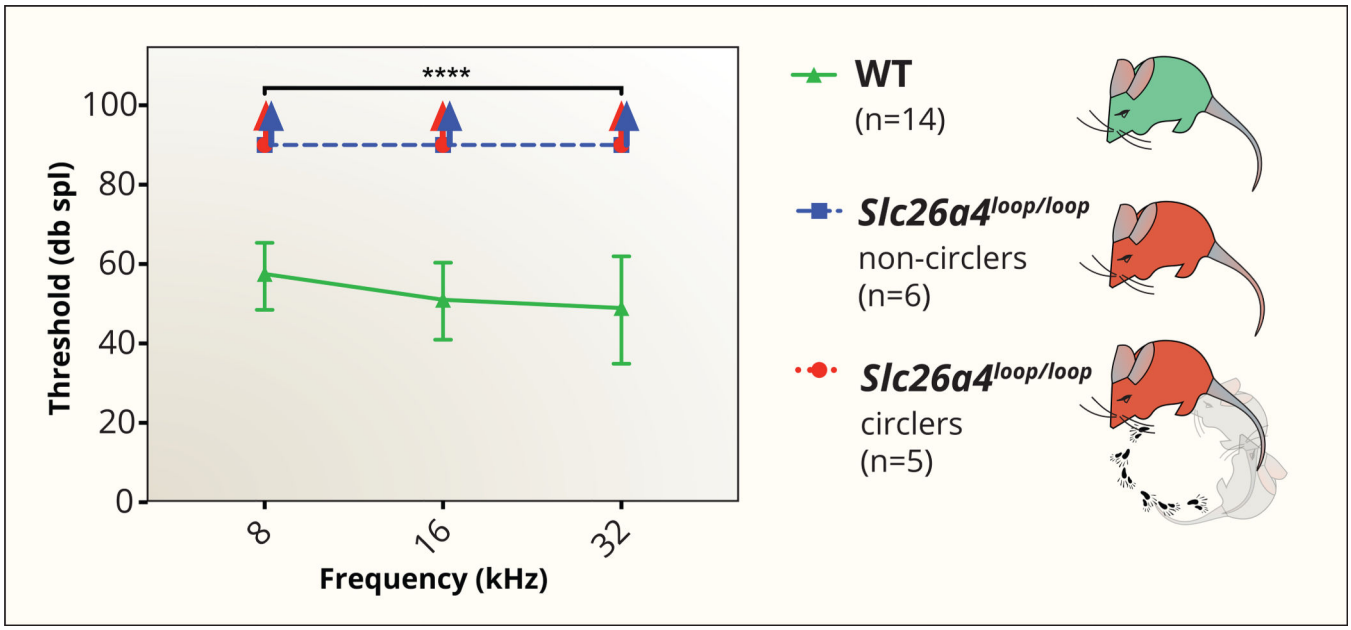


FIGURE 1.

The *Slc26a4*$^{loop/loop}$ mutant mice are profoundly deaf but show variable vestibular dysfunction. Tone-burst ABR tests demonstrate that *Slc26a4*$^{loop/loop}$ are all deaf with no exception and regardless of any vestibular abnormality.

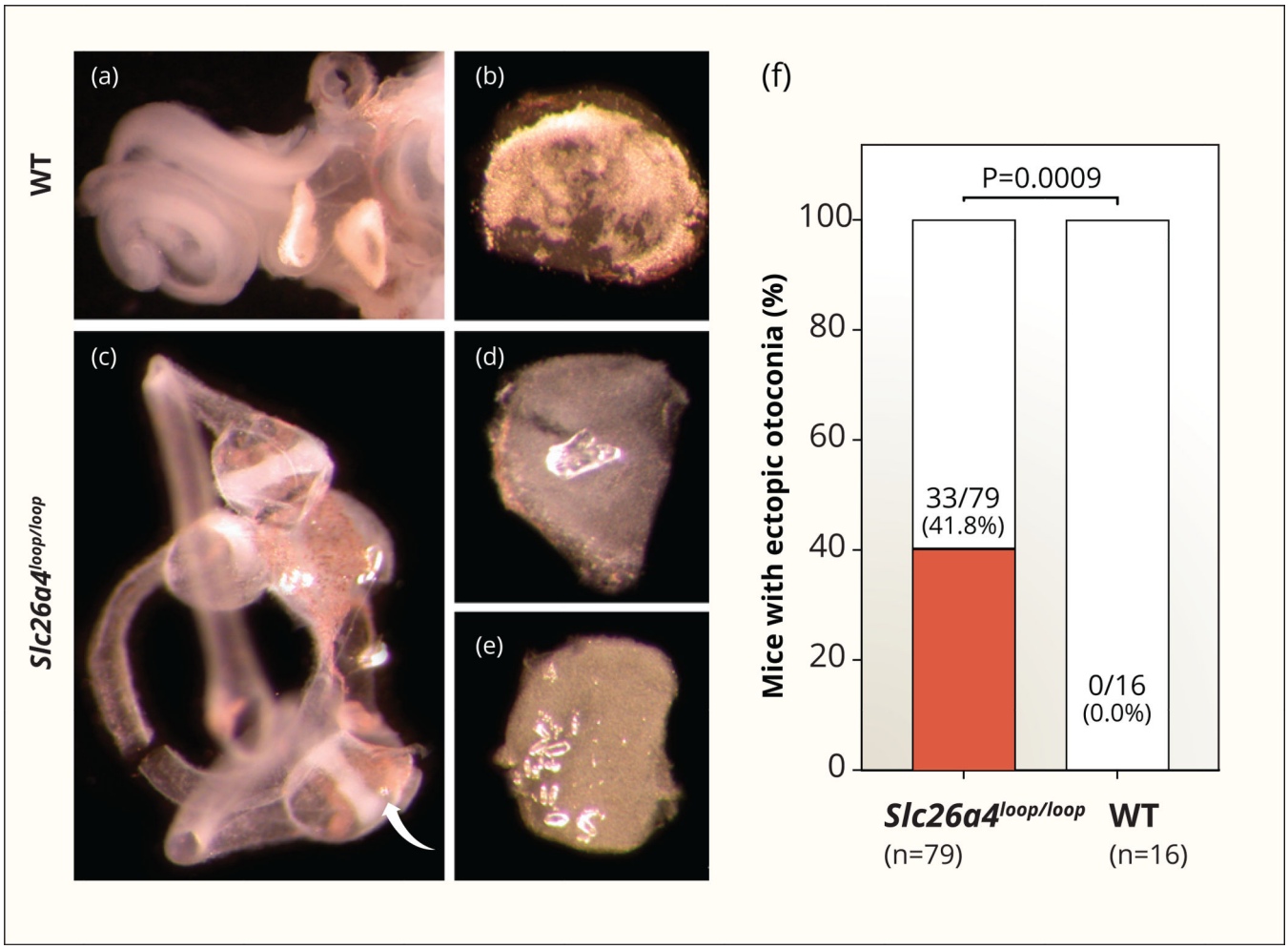
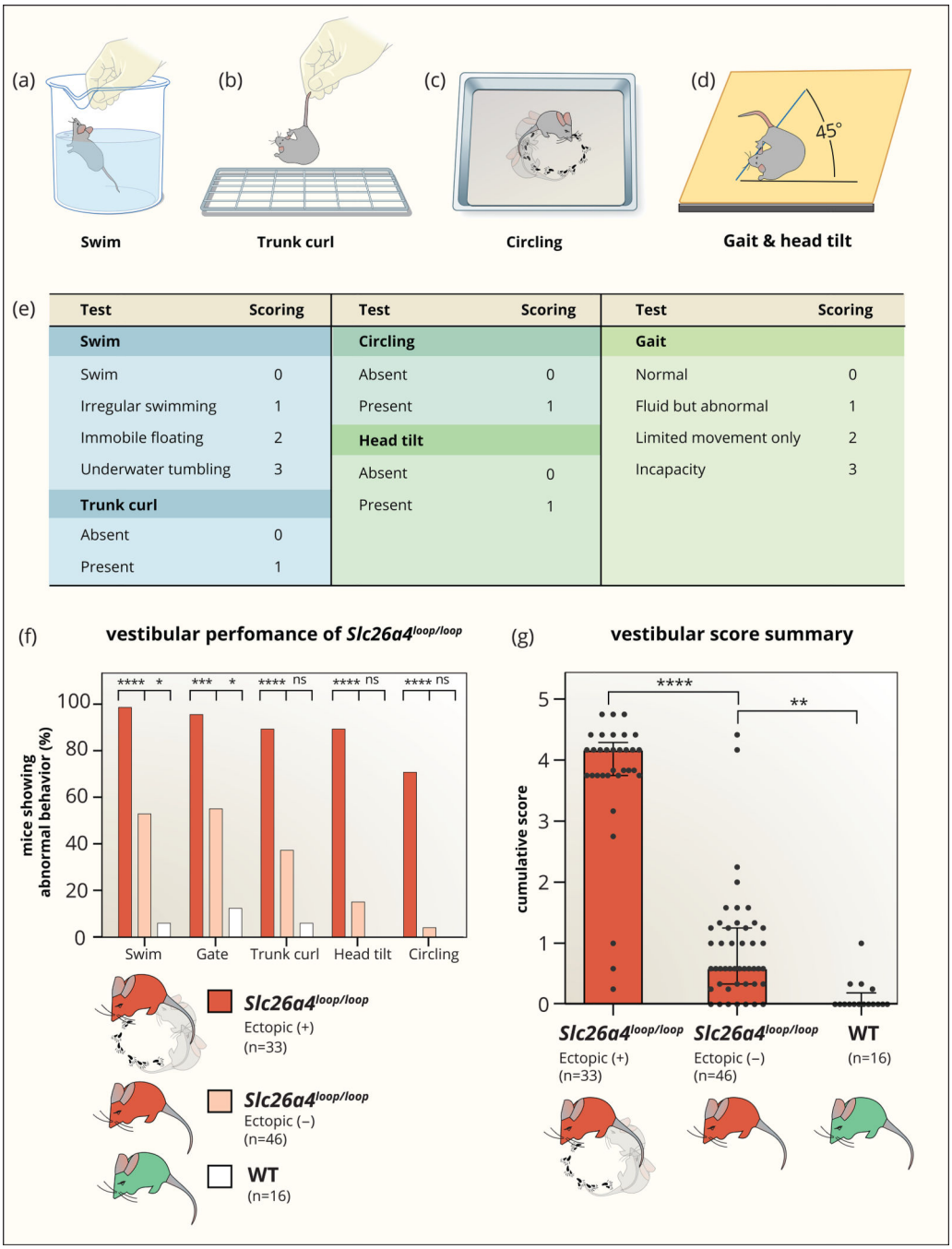


FIGURE 2.

A battery of behavioral tests distinguish between two subgroups of *Slc26a4*$^{loop/loop}$ mutant mice, based on variable vestibular dysfunction. Selected behavioral tests were used in the behavioral pipeline: (a) the forced swimming test; (b) the trunk curl test; (c) presence of circling behavior and/or head tilt; and (d) observation for gait abnormality. (e) The scoring system with a description for each individual test is depicted. A cumulative score was given to each mouse following the completion of all tests. (f) A significant difference between two subgroups of mutant mice with and without vestibular deficiency is shown. The average cumulative score of each group is shown for each behavioral test separately (column bar graph) and collectively (box plot), summarizing all behavioral tests score together. For mutants with vestibular dysfunction, N=32; without vestibular dysfunction, N=47.



Test	Scoring	Test	Scoring	Test	Scoring
Swim		**Circling**		**Gait**	
Swim	0	Absent	0	Normal	0
Irregular swimming	1	Present	1	Fluid but abnormal	1
Immobile floating	2	**Head tilt**		Limited movement only	2
Underwater tumbling	3	Absent	0	Incapacity	3
Trunk curl		Present	1		
Absent	0				
Present	1				

FIGURE 3.

Displaced otoconia strongly correlate with severe vestibular dysfunction of *Slc26a4*$^{loop/loop}$ mutant mice. (a, b) In wild-type mice, thousands of 5um otoconia are associated with the sensory macula of the utricle and saccule. (c) The vestibular membranous labyrinth of *Slc26a4*$^{loop/loop}$ mutant mice with severe vestibular dysfunction is shown. An ectopic giant otoconia is present at the posterior crista ampullaris (white arrow). (d, e) In all *Slc26a4*$^{loop/loop}$ mutant mice, giant otoconia were detected within the utricle and saccule. (f) Among the subgroup with severe vestibular dysfunction, an ectopic giant otoconia was

found in at least one ear in 30 out of 32 mutant mice (93.8%). In the subgroup of mutant mice with normal vestibular function, ectopic giant otoconia was found in only 3 out of 47 mice (6.4%). For mutant with vestibular dysfunction, N=32; and without vestibular dysfunction, N=47.

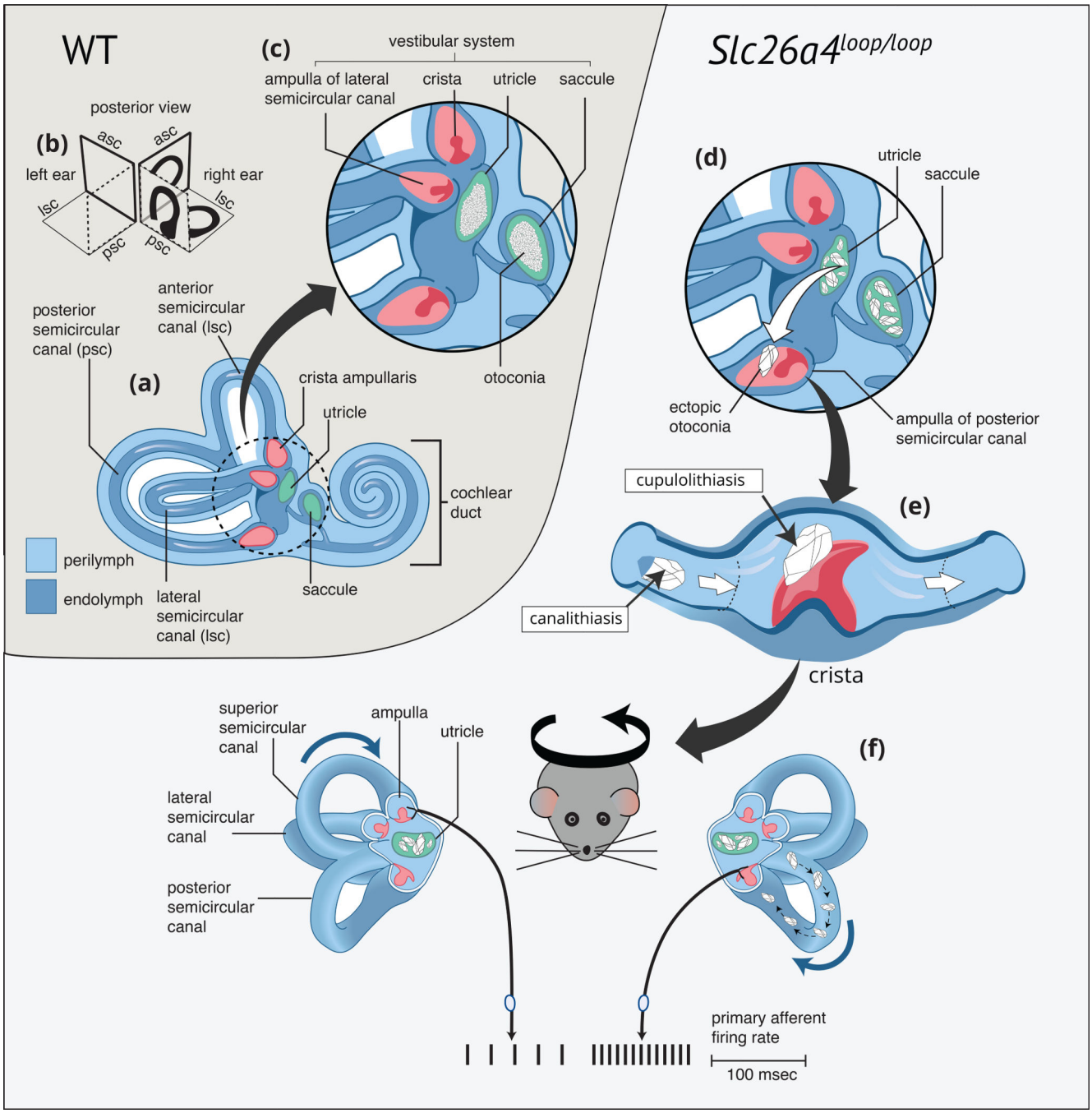


FIGURE 4.

Ectopic otoconia underlies severe vestibular dysfunction of *Slc26a4*$^{loop/loop}$ mutant mice. (a) The inner ear is divided into two functional anatomical units, the cochlear system for hearing and the vestibular system for balance and motion perception. The vestibule is comprised of five sensory organ, two maculae, utricle and saccule, for perception of gravity, linear and vertical acceleration (green). (b) The three cristae ampullaris and associated semicircular canals are responsible for perception of angular and rotational head movement in the three-dimensional (3D) axis. (c) In wild type mice, thousands of otoconia are

associated with the sensory maculae of the utricle and saccule (green). (d) In *Slc26a4*$^{loop/loop}$ mutant mice, otoconia are replaced with a giant mineralized body. (e) The abnormal otoconia particle is occasionally displaced from maculae, either floating in endolymph (canalithiasis) or attached to the gelatinous cupula of the cristae (cupulithiasis) and leads to pathological vestibular stimulation. (f) Under normal conditions, head rotation in one direction leads to inertia of endolymphatic fluids within the semicircular canal to the opposite direction. Depending on the direction of fluid inertia, deflection of the cupula either activates or suppresses the underlying sensory hair cells. An excitatory stimulus triggered by a semicircular canal in one ear is simultaneously coupled with inhibitory suppression of the contralateral semicircular canal corresponding to the same plan of rotational axis. The balance between primary afferent firing rates of both sides is processed towards a central vestibular sensation.