RESEARCH ARTICLE

alms1 mutant zebrafish do not show hair cell phenotypes seen in other cilia mutants

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Abstract

Multiple cilia-associated genes have been shown to affect hair cells in zebrafish (*Danio rerio)*, including the human deafness gene *dcdc2*, the radial spoke gene *rsph9*, and multiple intraflagellar transport (IFT) and transition zone genes. Recently a zebrafish *alms1* mutant was generated. The *ALMS1* gene is the gene mutated in the ciliopathy Alström Syndrome a disease that causes hearing loss among other symptoms. The hearing loss seen in Alström Syndrome may be due in part to hair cell defects as *Alms1* mutant mice show stereocilia polarity defects and a loss of hair cells. Hair cell loss is also seen in postmortem analysis of Alström patients. The zebrafish *alms1* mutant has metabolic defects similar to those seen in Alström syndrome and *Alms1* mutant mice. We wished to investigate if it also had hair cell defects. We, however, failed to find any hair cell related phenotypes in *alms1* mutant zebrafish. They had normal lateral line hair cell numbers as both larvae and adults and normal kinocilia formation. They also showed grossly normal swimming behavior, response to vibrational stimuli, and FM1-43 loading. Mutants also showed a normal degree of sensitivity to both short-term neomycin and long-term gentamicin treatment. These results indicate that cilia-associated genes differentially affect different hair cell types.

Introduction

Hearing and balance disorders are common sensory disorders [1, 2]. There is a significant genetic component to these disorders, with over 50% of congenital hearing loss seen in newborns being hereditary [3]. To date, 121 nonsyndromic hearing loss genes have been identified in addition to many syndromic hearing loss genes [4]. While most of these genes are only implicated in hearing loss, mutations in a small subset also cause balance disorders [5, 6]. Genetic mutations causing hearing loss can affect various processes important for hearing. A number of these mutations specifically affect the development and function of the sensory hair cells responsible for hearing [3].

Zebrafish have evolved as a useful model to study the genetics of hair cell function. Zebrafish are powerful genetic models and have hair cells on the surface of their bodies as part of the lateral line system, which allows for the observation of hair cells in an intact *in vivo* model. Researchers have carried out large-scale genetic screens to identify zebrafish mutants with auditory or vestibular defects [7–9] as well as generated zebrafish models of known deafness genes [10, 11].

One class of genes that have been shown to influence hair cells in humans and zebrafish are cilia-associated genes. Multiple ciliopathies, genetic disorders with mutations in cilia-associated genes, have been associated with hearing loss, including Usher Syndrome, Alström Syndrome, and Bardet-Biedl Syndrome (BBS) [12–16]. A number of Usher Syndrome genes have also been shown to affect hair cell function in zebrafish [17–22]. Other cilia genes associated with hearing loss in humans include *DCDC2*, *CDC14A*, *CCDC114*, and the basal body genes *CEP78* and *CEP250* [23–28]. While some of these genes, such as *dcdc2*, are similarly necessary for hair cell function in zebrafish [26] others such as *cdc14A* do not cause zebrafish hair cell phenotypes when mutated [29]. There have also been a number of cilia-associated genes that have not been implicated in human deafness but have been shown to cause hair cell phenotypes when disrupted in zebrafish. Mutations in intraflagellar transport (IFT) genes, genes necessary for the transport of proteins along cilia, in zebrafish have been shown to decrease hair cell numbers, lead to resistance to aminoglycoside-induced hair cell death, and to cause defects in FM1-43 uptake and early reverse polarity hair cell mechanotransduction activity [30–33]. Mutations in transition zone genes, genes that act as gatekeepers for proteins exiting and entering cilia, have also been shown to cause resistance to aminoglycoside-induced hair cell death but to have normal FM1-43 uptake and control hair cell numbers [32, 34], and a mutation in *rsph9*, a radial spoke gene in motile cilia, caused a reduced initiation of the startle response to acoustic stimuli in zebrafish suggesting a potential hair cell defect [35].

Recently a zebrafish *alms1* mutant line was generated [36]. *ALMS1* is the gene responsible for Alström Syndrome, a ciliopathy characterized by hearing and vision loss in addition to obesity and diabetes [13, 37, 38]. Dilated cardiomyopathy, hypertriglyceridemia, gastrointestinal disturbances, neurological symptoms, and liver and kidney dysfunction have also been observed in some patients [39]. ALMS1 localizes to the basal body at the base of cilia leading to the classification of Alström Syndrome as a ciliopathy [40]. Hearing loss in Alström Syndrome patients usually begins in childhood and is progressive [39, 41, 42] with patients also showing abnormal distortion product otoacoustic emissions (DPOAEs) [42, 43] Vestibular defects have not been observed. Postmortem analysis of patient auditory tissue shows degeneration of the organ of Corti, including a loss of hair cells. Additionally, there is atrophy of the stria vascularis and spiral ligament and degeneration of the spiral ganglion. In contrast to the auditory tissue, vestibular epithelium looked normal in these patients, in agreement with the lack of vestibular symptoms [44]. Mouse *Alms1* mutants show similar phenotypes as human patients, including obesity, retinal dysfunction, hyperinsulinemia, and delayed onset hearing loss [45–47]. Hair cells in *Alms1* mutant mice show misshapen stereocilia bundles and polarity defects and a loss of outer hair cells with age. Strial atrophy is also seen similar to what was observed in human postmortem samples [44, 47]. Similar to mouse mutants, zebrafish *alms1* mutants share many phenotypes with Alström Syndrome patients, including retinal degeneration, kidney and cardiac defects, increased fat disposition in the liver, a propensity for obesity, hyperinsulinemia, and glucose response defects [36]. However, hair cells and the auditory and vestibular systems of these fish have not been examined.

We wished to examine *alms1* mutant zebrafish to see if they showed hair cell phenotypes similar to mammalian *Alms1* mutants and other zebrafish cilia-associated gene mutants. We found that *alms1* mutant zebrafish had normal cilia formation in lateral line hair cells and other ciliated cells similar to what has been observed in mammalian *Alms1* mutants. We also failed to find any hair cell phenotypes in these mutants. They showed grossly normal audiovestibular behavior and sensitivity to aminoglycosides as both larvae and adults. They also showed normal FM1-43 uptake into hair cells. These results and the lack of vestibular defects seen in mammals following *Alms1* mutations suggest a specific role for *Alms1* in the auditory system rather than more global hair cell function. It also shows that cilia-associated genes have distinct roles in different hair cell types.

Materials and methods

Animals

Experiments used either 1 or 5 days post-fertilization (dpf) *Danio rerio* (zebrafish) larvae or adult zebrafish over three months of age. We used the previously described *alms1*^{umd2} mutant line, which causes a premature stop codon [36]. Mutant larvae were generated by either crossing two heterozygous animals together, or one heterozygous animal to a homozygous mutant, and comparing homozygous mutants to either wild-type or heterozygous siblings born at the same time. For the experiments on vibrational stimulus response and adult zebrafish, homozygous mutants were incrossed to generate the mutant animals, and homozygous wild-type siblings of those mutants were crossed to heterozygous wild-type siblings to generate the wild-type controls. Larvae were raised in embryo media (EM) consisting of 1 mM MgSO$_4$, 150 μM KH$_2$PO$_4$, 42 μM Na$_2$HPO$_4$, 1 mM CaCl$_2$, 500 μM KCl, 15 mM NaCl, and 714 μM NaHCO$_3$ and housed in an incubator maintained at 28.5°C with a 14/10 hour light/dark cycle. The Lafayette College Institution Animal Care and Use Committee approved all experiments.

Antibody labeling, imaging, and cilia length measurement

Zebrafish larvae and fins used for antibody labeling were fixed for two hours at room temperature in 4% paraformaldehyde. Antibody labeling was carried out as previously described [48]. Cilia were labeled with a mouse anti-acetylated tubulin primary antibody (Millipore-Sigma, T7451) diluted at 1:1,000 in antibody block. Larvae used for hair cell counts were labeled with a rabbit anti-parvalbumin primary antibody (ThermoFisher, PA1-933) diluted at 1:1,000 in antibody block. Fins used for hair cell counts were labeled with both the parvalbumin antibody and a mouse anti-otoferlin antibody (Developmental Studies Hybridoma Bank, HCS-1) diluted at 1:100 in antibody block.

Images of antibody labelled larvae and fins were taken on a Zeiss LSM800 confocal microscope. A stack of images 1 μm apart were taken throughout the tissue of interest and then maximum projection images of these stacks were generated for inclusion in the figures. To measure cilia length we chose a representative cilia that we could easily trace in each image and measured the length using Fiji.

Vibrational stimulus response

To test if *alms1* mutants would respond to vibrational stimuli we tapped a petri dish containing 50 *alms1* mutants with a glass pipette and recorded their response with an iPhone SE. To quantify this behavior we used a modified version of methods previously described [49]. Fish were moved into a fresh petri dish 8 fish at a time. After giving the fish a few minutes to acclimate to the new dish the dish was taped with a glass pipette three times a few seconds apart while recording the response of the fish with an iPhone SE. Videos were then scored with each fish given a score of 0 for no movement in response to the tap and a score of 1 if they moved in response to the tap. Fish that were showing spontaneous movement at the times of any of the taps or that were on the edge of the dish during the experiment were excluded. An average score across the three experiments was generated for each fish.

FM1-43 uptake

Fish were treated with 2.25 μM FM 1-43FX (ThermoFisher, F35355) for 1 minute in EM, washed 3 times in EM, and then anesthetized with MS-222/Tricaine-S (Pentair Aquatic Eco-Systems, TRS1) for imaging. Fish were imaged on a Zeiss LSM800 confocal microscope. For each fish, a single neuromast was imaged by taking a stack of 5 1 μm optical sections. Image

analysis was carried out in Fiji. A maximum projection image was made of each stack of images, and the average fluorescent intensity of the cell bodies of the entire neuromast was measured. Additionally, the average fluorescent intensity of an area in the background of the image was measured. Finally, the value for the fluorescence of the neuromast was divided by the value of the background fluorescence. Larvae were euthanized and genotyped after imaging.

Aminoglycoside treatment and hair cell counts

Fish were treated with either neomycin solution (Sigma-Aldrich, N1142) or gentamicin solution (Sigma-Aldrich G1272) at the indicated doses diluted in EM. For all neomycin treatment experiments, fish were treated with neomycin for 30 minutes, washed 3 times in either plain EM for larvae or plain system water for adult fish, left to recover in the third wash for one hour, and then tissue was collected for antibody labeling. In the case of larvae, whole larvae were fixed, whereas for adult fish, the fish were anesthetized, and their caudal fins were amputated and fixed. For gentamicin treatment, larvae were treated with gentamicin for 6 hours, washed 3 times in plain EM, and then fixed for antibody labeling. For adult zebrafish, 6 neuromasts on each fin were counted using the HCS-1 stain, and an average hair cell/neuromast number was generated for each animal. For larvae, the OP1, M2, IO4, MI2, and MI1 neuromasts [50] were counted using the parvalbumin stain, and again an average number of hair cells/neuromast number was generated for each animal. Larvae were genotyped after counting was complete. All hair cell counts were carried out on an Accu-Scope EXC-350 microscope.

Statistical analysis

All statistics were calculated using GraphPad Prism software (version 6.0).

Results

Cilia morphology is normal in *alms1* mutants

There have previously been conflicting results regarding whether ALMS1 plays a role in cilia formation and maintenance. Cilia in *Alms1* mutant mice and fibroblasts isolated from Alström syndrome patients appear grossly normal [40, 46, 51–53] even when there is no visible antibody labeling for ALMS1 protein [52]. However, RNAi knockdown of *Alms1* in cultured cells results in abnormal and stunted cilia [53, 54]. To test what cilia morphology looked like in zebrafish *alms1* mutants, we stained 24 hours post fertilization (hpf) and 5dpf zebrafish larvae with acetylated tubulin. We found that cilia morphology looked grossly normal in both early hair cells of the otic vesicle, hair cells of the lateral line and cells of the olfactory pit in *alms1* mutants (Fig 1) We also failed to see any early hair cells of the otic vesicle or neuromasts that were missing cilia or obvious gaps of missing cilia in the olfactory pit. Measuring cilia length we did not see any significant differences in the average cilia length in these tissues. We did however, observe an increase in the variability of cilia length in *alms1* mutants in the early hair cells in the otic vesicle (Fig 1).

alms1 mutants do not show mechanotransduction defect phenotypes

Zebrafish mechanotransduction mutants show several characteristic behavioral phenotypes, including a failure to remain upright, circling behavior when swimming, and a failure to respond to acoustic-vibrational stimuli [9]. It has previously been shown that zebrafish morphants of another cilia-associated deafness gene, *dcdc2*, show similar behavioral defects [26]. We, however, failed to observe any of these phenotypes in *alms1* mutant zebrafish. *alms1* 5dpf
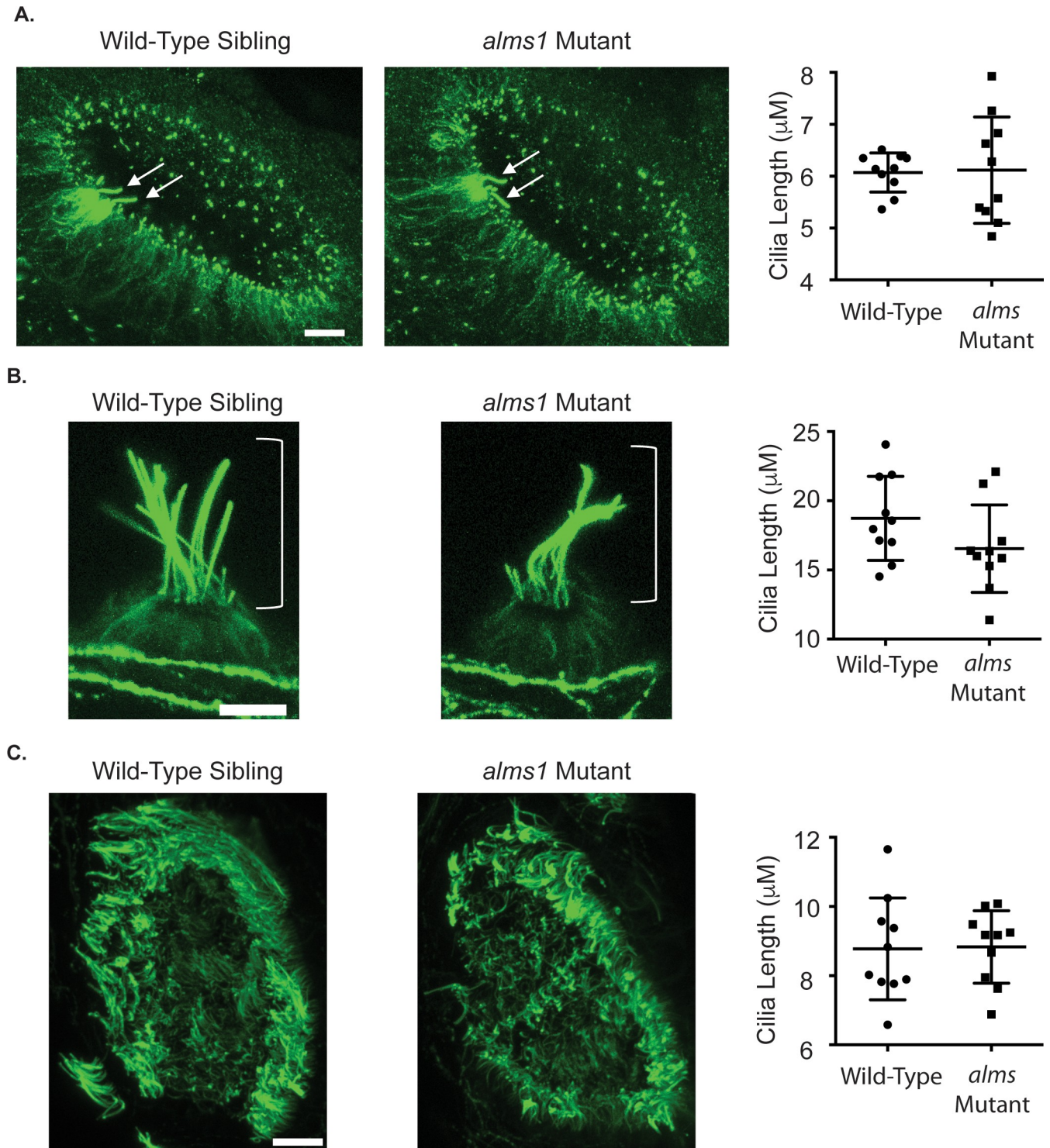
Fig 1. Cilia formation is largely normal in *alms1* mutants. (A) Representative images of the otic vesicle of a 24 hpf wild-type (left) and *alms1* mutant (center) zebrafish embryo stained with acetylated tubulin. The arrows point to the cilia of the early hair cells. To the right is quantification of cilia length. There was no significant difference in average kinocilia length between wild-type and mutant zebrafish, however, there was more variation in length seen in *alms1* mutants. (p = 0.0065 by F test to compare variances) (B) Representative images of the IO1 neuromast stained with acetylated tubulin in wild-type (left) and *alms1* mutant (center) 5dpf zebrafish larvae. The brackets show the position of the kinocilia. Kinocilia appear grossly normal in *alms1* mutant fish. To the right is quantification of IO1 kinocilia length. There was no significant difference between wild-type and mutant zebrafish (p = 0.1312 by an unpaired t-test) (C) Representative images of the olfactory pit stained with acetylated tubulin in wild-type (left) and *alms1* mutant (center) 5dpf zebrafish larvae. Again cilia appear

grossly normal in *alms1* mutants. To the right is quantification of cilia length in the olfactory pit. There was no significant difference between wild-type and mutant zebrafish (p = 0.9247 by unpaired t-test). Scale bar for all images = 10 μm. Quantification graphs show individual data points along with the mean and standard deviation of the data, n = 10 fish per group.

mutant larvae remain upright, and those without body morphology defects showed a grossly normal escape response following a vibrational stimulus generated by taping on their dish (S1 Movie and Fig 2). Adult homozygous mutants also showed normal swimming behavior (S2 Movie). The rapid uptake of FM1-43 is also reduced or eliminated when hair cell mechano-transduction activity is impaired [55–57]. It has previously been shown that other cilia gene mutants, specifically IFT mutants, show reduced levels of FM1-43 loading into lateral line hair cells [32, 33]. However, we failed to observe any significant differences in FM1-43 loading in *alms1* mutants compared to wild-type siblings (Fig 3).

alms1 mutants are not resistant to aminoglycoside-induced hair cell death

It has previously been shown that some zebrafish cilia-associated gene mutants are resistant to aminoglycoside-induced hair cell death, specifically transition zone, including the basal body gene *cep290*, and IFT gene mutants [32–34]. To test if this was also the case for *alms1* mutants, we used both short-term neomycin and long-term gentamicin treatment paradigms and
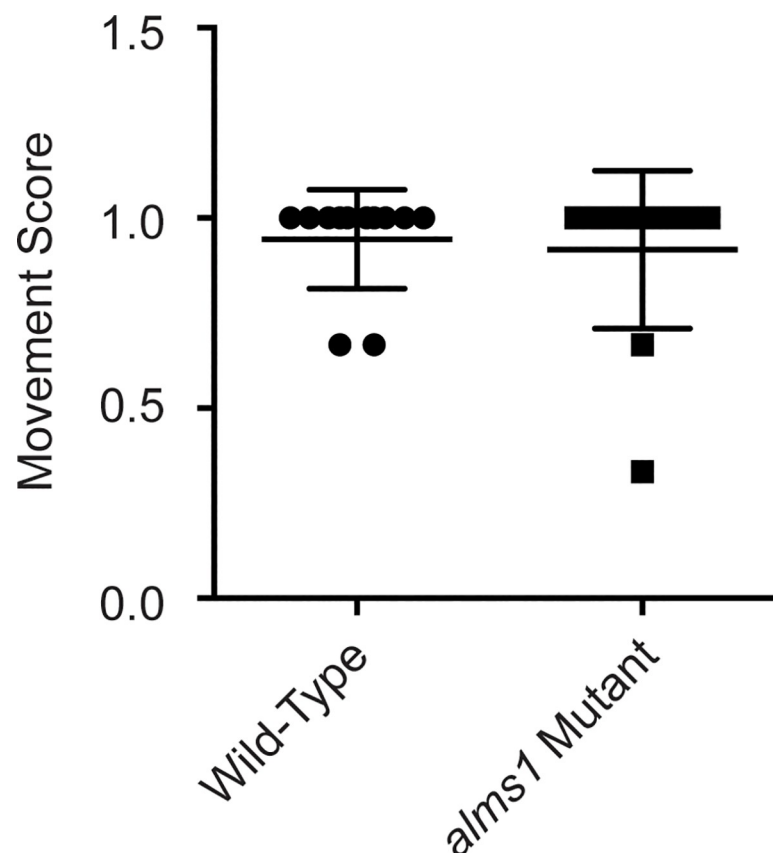

Fig 2. *alms1* mutants respond to vibrational stimuli. Quantification of the number of times fish respond to a vibrational stimuli across three times. A score of 1 means a fish responded all three times whereas a score of 0 means a fish never responded. There was no significant difference between the two groups by an unpaired t-test (p = 0.6977), n = 12 fish per group. Individual data points are shown along with the mean and standard deviation of the data.
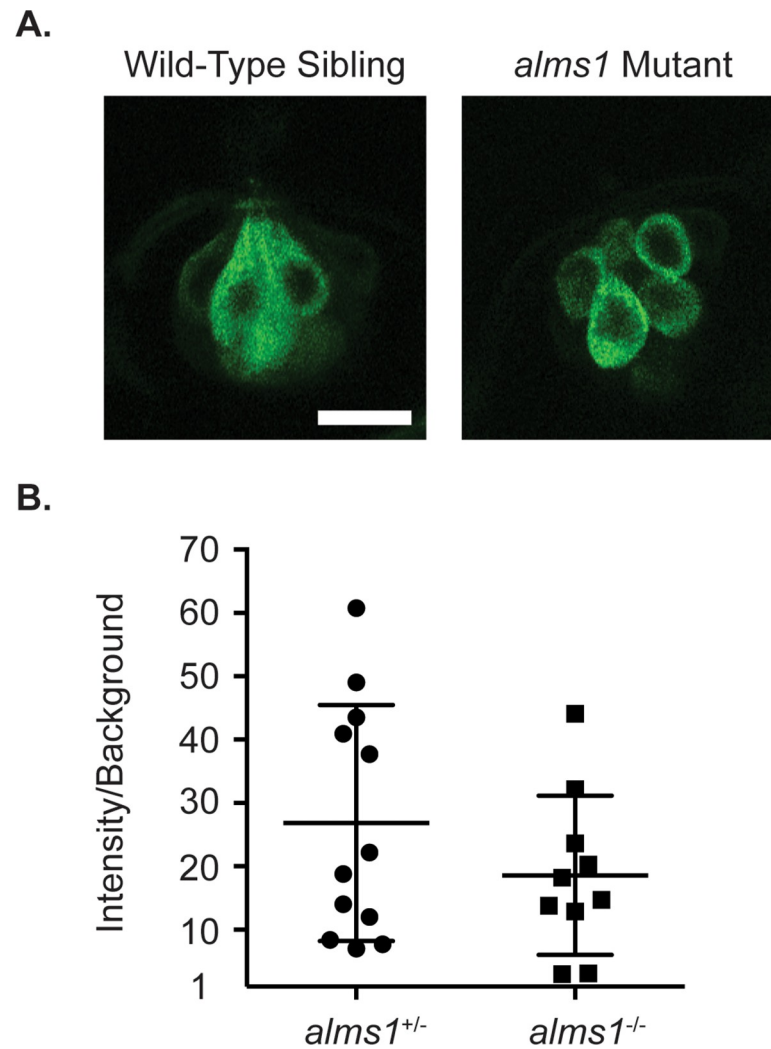
A.

B.

Fig 3. FM1-43 loading is normal in *alms1* mutants. (A) Representative images of neuromasts from wild-type siblings (left) and *alms1* mutants (right) treated with FM1-43. Scale bar = 10 μm. (B) Quantification of the fluorescent intensity of FM1-43 in neuromasts of heterozygous wild-type siblings and homozygous *alms1* mutants. There was no significant difference between the two groups by an unpaired t-test (p = 0.2474). Individual data points are shown along with the mean and standard deviation of the data.

quantified hair cell number in 5dpf zebrafish larvae. We failed to see any significant differences between wild-type mutants and homozygous mutants in either control conditions or following aminoglycoside treatment (Fig 4A–4C). Many cilia-associated genes are maternally expressed in zebrafish, meaning a heterozygous mother loads wild-type RNA into the egg, and this can mask mutant phenotypes early in development [58–60]. Therefore, we wished to test if this was happening in *alms1* mutants by generating mutant larvae out of both heterozygous and homozygous mutant mothers and treating them with neomycin. We again failed to find any significant differences between the different genotypes (Fig 4B).

In human Alström syndrome, patients' hearing loss is usually not seen until later in childhood [39, 42, 61]. Likewise, hearing defects in *Alms1* mutant mice show a delayed onset [45–47]. Therefore, we wished to see if adult zebrafish were resistant to aminoglycoside-induced hair cell death even though larvae were not. We treated fish slightly over 3 months of age that
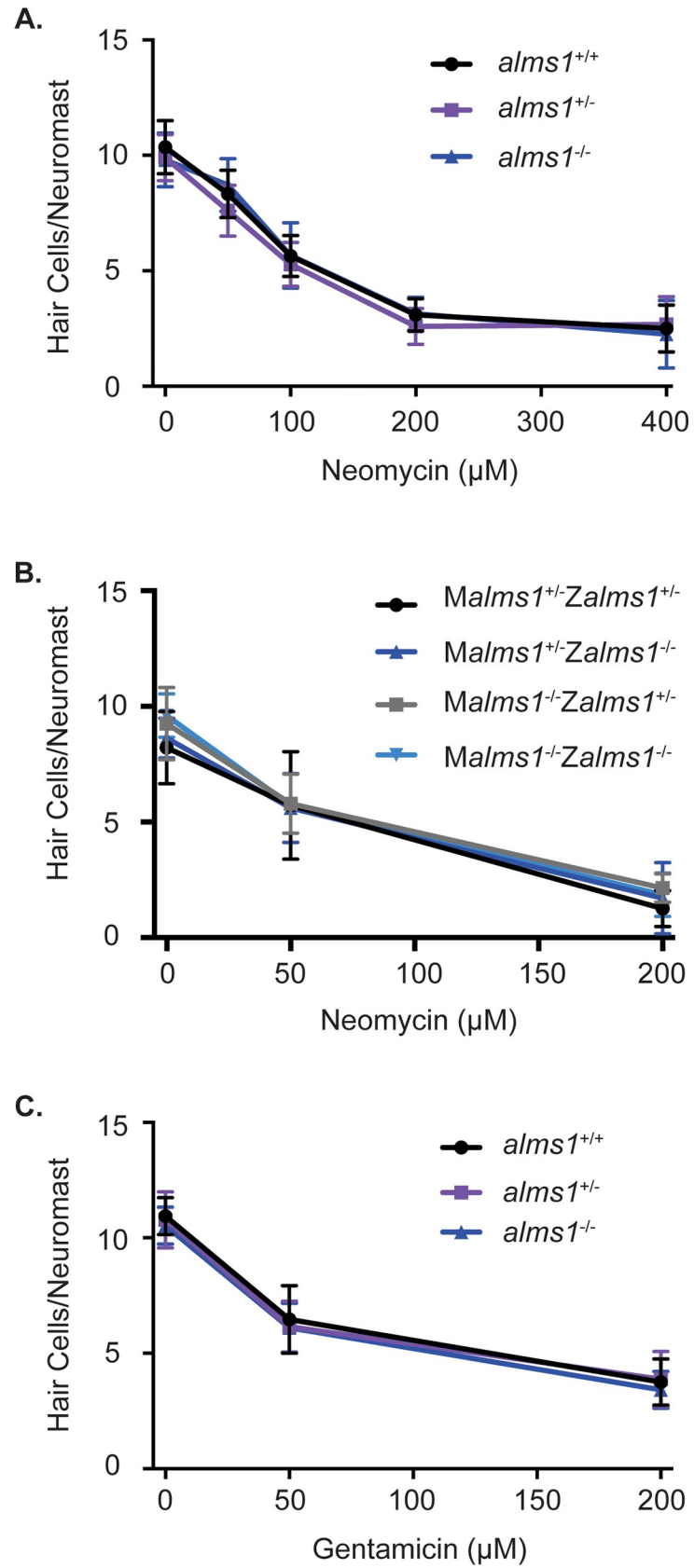
Fig 4. *alms1* mutant larvae are not resistant to aminoglycoside-induced hair cell death. (A) Dose-response curve for wild-type siblings and *alms1* mutants from an incross of heterozygous parents treated with 0–400 μM neomycin for 30 minutes with a one-hour recovery time. n = 7–19 fish per neomycin dose for homozygous wild-type and mutants and 20–25 for heterozygous fish. There were no significant differences due to genotype (p = 0.1273) or the interaction between genotype and neomycin (p = 0.3939) by two-way ANOVA. (B) Dose-response curve for fish that were either heterozygous or homozygous for the *alms1* mutation from mothers also either heterozygous or homozygous for the *alms1* mutation treated with 0–200 μM neomycin for 30 minutes with a one-hour recovery time. M = maternal genotype and Z = zygotic genotype. n = 7–16 fish per neomycin dose for each genotype. There were no significant differences due to genotype (p = 0.8060) or the interaction between genotype and neomycin (p = 0.2024) by two-way ANOVA. (C) Dose-response curve for wild-type siblings and *alms1* mutants from parents heterozygous for the *alms1* mutation treated with 0–200 μM gentamicin for 6 hours. n = 10–14 fish per gentamicin dose for homozygous wild-type and mutants and 21–25 for heterozygous fish. There were no significant differences due to genotype (p = 0.9401) or the interaction between genotype and neomycin (p = 0.2850) by two-way ANOVA. Data are shown as mean +/- standard deviation.

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appeared to be mature adults (S2 Movie) with neomycin. We again failed to find any significant difference between homozygous mutants and related wild-type fish in either control lateral line hair cell numbers or hair cell numbers following neomycin treatment (Fig 5).

Discussion

Zebrafish have emerged as a useful model to study hair cell function and death in part due to the external location of hair cells in the lateral line system. Multiple genes associated with both syndromic and nonsyndromic forms of deafness have been shown to cause hair cell related phenotypes in zebrafish [10, 11]. This is particularly true for genes affecting mechanotransduction or synaptic activity [62, 63]. However, not all deafness genes have been shown to have phenotypes when mutated in zebrafish, including the cilia-associated gene *cdc114* [29]. We found this also to be the case with *alsm1*. *alms1* has previously been shown to be expressed in hair cells of zebrafish larvae and adults [64, 65] as well as showing up in the mature lateral line hair cell cluster in single cell RNA-Seq data [66]. Despite this we noticed no obvious hair cell phenotypes in *alms1* zebrafish mutants. Fish showed grossly normal swimming behavior and response to vibrational stimuli, as well as normal hair cell number in contrast to what has previously been shown in mouse *Alms1* mutants [47] and human patients [44]. These mutants also showed normal FM1-43 uptake and aminoglycoside toxicity of lateral line hair cells in contrast to what has been seen in other zebrafish cilia-associated gene mutants [32–34].

There are multiple explanations for the lack of phenotypes we observed. First, it has previously been shown that CRISPR mutants can show genetic compensation that is not present when genes are merely knocked down [67]. It is also possible that the lack of phenotype we observe is due to a lack of sequence conservation between the mammalian and zebrafish *alms1* gene. The majority of the N-terminus of the human *ALMS1* gene, encoding for the tandem repeat domain of the protein, is specific to mammals [68, 69] with the first 1560 amino acids of the human gene having no homologous region in the zebrafish gene. However, as the zebrafish mutant shows multiple expected phenotypes due to defects in other tissues [36] if any of these issues were responsible for the lack of phenotype we see, it would have to be relatively hair cell specific. It is also possible that *alms1* has an ohnologue compensating for its loss in hair cells. Teleost fish underwent a genome duplication event [70, 71] and other zebrafish hair cell gene mutants have failed to completely phenocopy mammalian mutants due to either ohnologue specific functions or restricted expression of ohnologues [19, 72–74]. *alms1*, however, does not have any known ohnologues in zebrafish [75] and performing a protein blast for the human and zebrafish *alms1* gene as well as the ALMS protein motif did not identify any candidate ohnologues. This blast did show the presence of a *C10orf90* homologue in zebrafish, which is a known centrosomal gene with an ALMS motif [37, 68], however, this gene is also present in
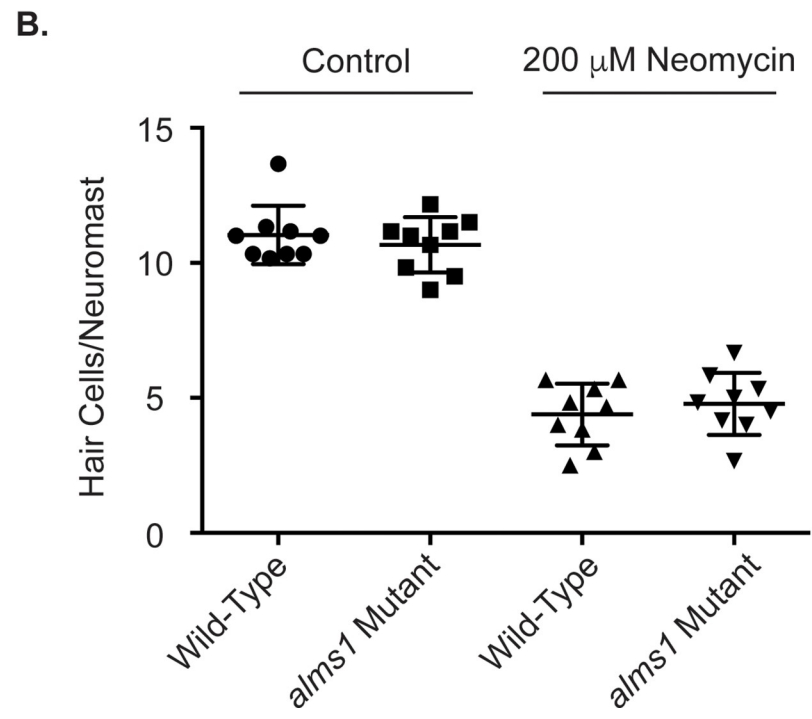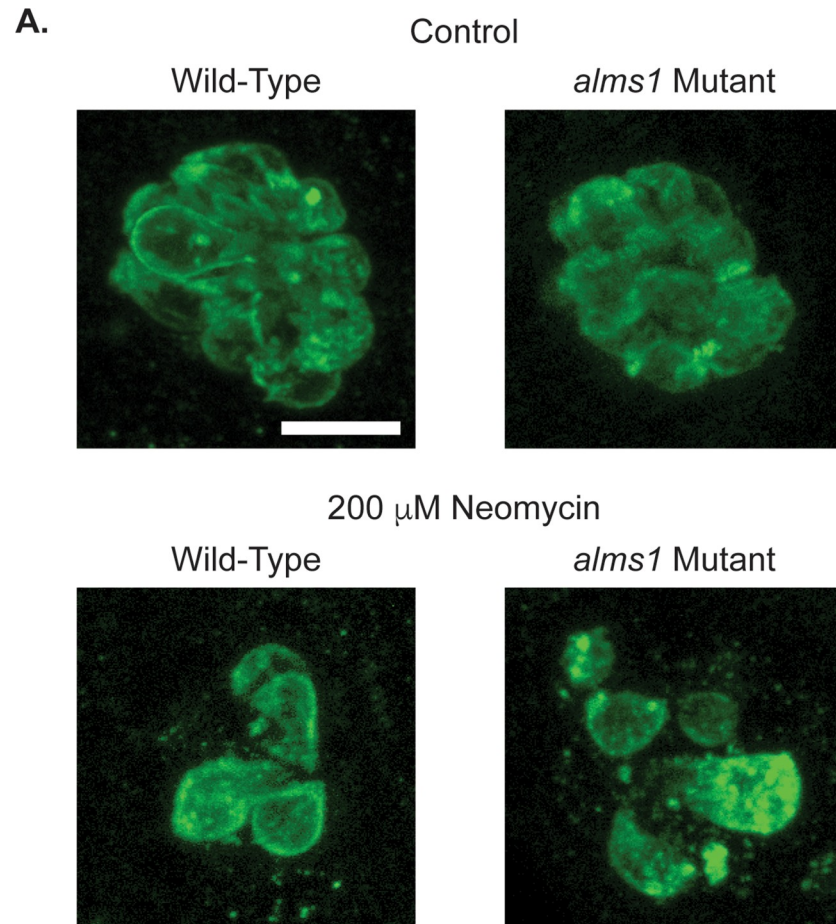
A.

Control

Wild-Type   *alms1* Mutant

200 μM Neomycin

Wild-Type   *alms1* Mutant

B.

Fig 5. Adult *alms1* mutant fish are not resistant to neomycin-induced hair cell death. (A) Representative images of neuromasts from control (top) and neomycin treated (bottom) fish from wild-type (left) and *alms1* mutant (right) fish. (B) Quantification of hair cells/neuromast either in control conditions or following treatment with 200 μM neomycin. Individual data points are shown along with the mean and standard deviation. There were no significant differences due to genotype (p = 0.3073) or the interaction between genotype and neomycin (p = 0.9784) by two-way ANOVA.

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mammals and is not able to compensate for *alms1* defects there. We can also not rule out more subtle defects, particularly in inner ear hair cells, that were not observed by the methods we used.

However, we feel the most likely explanation for the lack of observed phenotypes is different roles for *alms1* in different hair cell types. While human Alström patients and *Alms1* mutant mice show hearing defects and defects in auditory hair cells, there have not been any reported vestibular defects. ALMS1 specifically localizes to the centrosome or basal body of cilia [40, 47] and other basal body proteins have likewise been associated with hearing loss but not vestibular dysfunction [23, 24]. While different hair cell types have many shared genes, there are also many genes that are auditory or vestibular hair cell specific [76, 77]. Comparing lists of highly expressed zebrafish hair cell genes [64, 65] to these lists show overlap with several vestibular hair cell specific genes but no auditory hair cell specific genes. This suggests zebrafish hair cells are more closely related to mammalian vestibular hair cells than auditory hair cells. One of the defects seen in *Alms1* mutant hair cells in mammals, is stereocilia polarity [47]. This defect has also been shown in auditory hair cells of other cilia-associated gene mutants [15, 78–80]. However, in some cases, these mutants had no polarity defects in vestibular hair cells [79, 80] and these polarity defects have not been seen in lateral line hair cells of zebrafish cilia-associated gene mutants [30, 32]. Therefore, it appears that in mammalian auditory hair cells where the kinocilia, the true cilia of the cell, is lost early in development [81] the primary role of cilia-associated genes has evolved to be determining stereocilia polarity early in development. Whereas, in mammalian vestibular hair cells and zebrafish hair cells, where the kinocilia are maintained, these genes may play other functions that are not yet fully elucidated.

Supporting information

S1 Movie. alms1 mutant larvae's response to vibrational stimuli.
(MP4)

S2 Movie. Adult alms1 mutants.
(MP4)

S1 Data.
(ZIP)

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Supervision: Tamara M. Stawicki.

Visualization: Tamara M. Stawicki.

Writing – original draft: Tamara M. Stawicki.

Writing – review & editing: Tamara M. Stawicki.

References

1. Goman AM, Lin FR. Prevalence of Hearing Loss by Severity in the United States. Am J Public Health. 2016; 106: 1820–1822. https://doi.org/10.2105/AJPH.2016.303299 PMID: 27552261

2. Agrawal Y, Carey JP, Della Santina CC, Schubert MC, Minor LB. Disorders of balance and vestibular function in US adults: Data from the National Health and Nutrition Examination Survey, 2001–2004. Arch Intern Med. 2009; 169: 938–944. https://doi.org/10.1001/archinternmed.2009.66 PMID: 19468085

3. Delmaghani S, El-Amraoui A. Inner Ear Gene Therapies Take Off: Current Promises and Future Challenges. J Clin Med. 2020; 9: 2309. https://doi.org/10.3390/jcm9072309 PMID: 32708116

4. Van Camp G, Smith R. Hereditary Hearing Loss Homepage. [cited 1 Nov 2020]. Available: https://hereditaryhearingloss.org/

5. Eppsteiner RW, Smith RJH. Genetic disorders of the vestibular system. Curr Opin Otolaryngol Head Neck Surg. 2011; 19: 397–402. https://doi.org/10.1097/MOO.0b013e32834a9852 PMID: 21825995

6. Gallego-Martinez A, Espinosa-Sanchez JM, Lopez-Escamez JA. Genetic contribution to vestibular diseases. Journal of Neurology. Dr. Dietrich Steinkopff Verlag GmbH and Co. KG; 2018. pp. S29–S34. https://doi.org/10.1007/s00415-018-8842-7 PMID: 29582143

7. Malicki J, Schier AF, Solnica-Krezel L, Stemple DL, Neuhauss SCF, Stainier DYR, et al. Mutations affecting development of the zebrafish ear. Development. 1996; 123: 275–283. https://doi.org/10.5167/uzh-222 PMID: 9007247

8. Whitfield TT, Granato M, Van Eeden FJM, Schach U, Brand M, Furutani-Seiki M, et al. Mutations affecting development of the zebrafish inner ear and lateral line. Development. 1996; 123: 241–254. PMID: 9007244

9. Nicolson T, Rüsch A, Friedrich RW, Granato M, Ruppersberg JP, Nüsslein-Volhard C. Genetic Analysis of Vertebrate Sensory Hair Cell Mechanosensation: the Zebrafish Circler Mutants. Neuron. 1998; 20: 271–283. https://doi.org/10.1016/s0896-6273(00)80455-9 PMID: 9491988

10. Blanco-Sánchez B, Clément A, Phillips JB, Westerfield M. Zebrafish models of human eye and inner ear diseases. Methods Cell Biol. 2017; 138: 415–467. https://doi.org/10.1016/bs.mcb.2016.10.006 PMID: 28129854

11. Vona B, Doll J, Hofrichter MAH, Haaf T, Varshney GK. Small fish, big prospects: using zebrafish to unravel the mechanisms of hereditary hearing loss. Hear Res. 2020; 397: 107906. https://doi.org/10.1016/j.heares.2020.107906 PMID: 32063424

12. Reiners J, Nagel-Wolfrum K, Jürgens K, Märker T, Wolfrum U. Molecular basis of human Usher syndrome: deciphering the meshes of the Usher protein network provides insights into the pathomechanisms of the Usher disease. Exp Eye Res. 2006; 83: 97–119. https://doi.org/10.1016/j.exer.2005.11.010 PMID: 16545802

13. Alstrom C, Hallgren B, Nilsson L, Asander H. Retinal degeneration combined with obesity, diabetes mellitus and neurogenous deafness: a specific syndrome (not hitherto described) distinct from the Laurence-Moon-Bardet-Biedl syndrome: a clinical, endocrinological and genetic examination based on a lar. Acta Psychiatr Neurol Scand Suppl. 1959; 129: 1–35. PMID: 13649370

14. Girard D, Petrovsky N. Alström syndrome: Insights into the pathogenesis of metabolic disorders. Nature Reviews Endocrinology. Nat Rev Endocrinol; 2011. pp. 77–88. https://doi.org/10.1038/nrendo.2010.210 PMID: 21135875

15. Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, Jagger DJ, et al. Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. Nat Genet. 2005; 37: 1135–1140. https://doi.org/10.1038/ng1644 PMID: 16170314

16. Esposito G, Testa F, Zacchia M, Crispo AA, Di Iorio V, Capolongo G, et al. Genetic characterization of Italian patients with Bardet-Biedl syndrome and correlation to ocular, renal and audio-vestibular phenotype: Identification of eleven novel pathogenic sequence variants. BMC Med Genet. 2017; 18: 10. https://doi.org/10.1186/s12881-017-0372-0 PMID: 28143435

17. Ernest S, Rauch GJ, Haffter P, Geisler R, Petit C, Nicolson T. Mariner is defective in myosin VIIA: A zebrafish model for human hereditary deafness. Hum Mol Genet. 2000; 9: 2189–2196. https://doi.org/10.1093/hmg/9.14.2189 PMID: 10958658

18. Söllner C, Rauch G-J, Siemens J, Geisler R, Schuster SC, Müller U, et al. Mutations in cadherin 23 affect tip links in zebrafish sensory hair cells. Nature. 2004; 428: 955–959. https://doi.org/10.1038/nature02484 PMID: 15057246

19. Seiler C, Finger-Baier KC, Rinner O, Makhankov Y V., Schwarz H, Neuhauss SCF, et al. Duplicated genes with split functions: Independent roles of protocadherin 15 orthologues in zebrafish hearing and vision. Development. 2005; 132: 615–623. https://doi.org/10.1242/dev.01591 PMID: 15634702

20. Riazuddin S, Belyantseva IA, Giese APJ, Lee K, Indzhykulian AA, Nandamuri SP, et al. Alterations of the CIB2 calcium-and integrin-binding protein cause Usher syndrome type 1J and nonsyndromic deafness DFNB48. Nat Genet. 2012; 44: 1265–1271. https://doi.org/10.1038/ng.2426 PMID: 23023331

21. Ogun O, Zallocchi M. Clarin-1 acts as a modulator of mechanotransduction activity and presynaptic ribbon assembly. J Cell Biol. 2014; 207: 375–91. https://doi.org/10.1083/jcb.201404016 PMID: 25365995

22. Ebermann I, Phillips JB, Liebau MC, Koenekoop RK, Schermer B, Lopez I, et al. PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome. J Clin Invest. 2010; 120: 1812–1823. https://doi.org/10.1172/JCI39715 PMID: 20440071

23. Khateb S, Zelinger L, Mizrahi-Meissonnier L, Ayuso C, Koenekoop RK, Laxer U, et al. A homozygous nonsense CEP250 mutation combined with a heterozygous nonsense C2orf71 mutation is associated with atypical Usher syndrome. J Med Genet. 2014; 51: 460–469. https://doi.org/10.1136/jmedgenet-2014-102287 PMID: 24780881

24. Nikopoulos K, Farinelli P, Giangreco B, Tsika C, Royer-Bertrand B, Mbefo MK, et al. Mutations in CEP78 Cause Cone-Rod Dystrophy and Hearing Loss Associated with Primary-Cilia Defects. Am J Hum Genet. 2016; 99: 770–776. https://doi.org/10.1016/j.ajhg.2016.07.009 PMID: 27588451

25. Namburi P, Ratnapriya R, Khateb S, Lazar CH, Kinarty Y, Obolensky A, et al. Bi-allelic Truncating Mutations in CEP78, Encoding Centrosomal Protein 78, Cause Cone-Rod Degeneration with Sensorineural Hearing Loss. Am J Hum Genet. 2016; 99: 777–784. https://doi.org/10.1016/j.ajhg.2016.07.010 PMID: 27588452

26. Grati M, Chakchouk I, Ma Q, Bensaid M, DeSmidt A, Turki N, et al. A missense mutation in DCDC2 causes human recessive deafness DFNB66, likely by interfering with sensory hair cell and supporting cell cilia length regulation. Hum Mol Genet. 2015; 24: 2482–2491. https://doi.org/10.1093/hmg/ddv009 PMID: 25601850

27. Delmaghani S, Aghaie A, Bouyacoub Y, El Hachmi H, Bonnet C, Riahi Z, et al. Mutations in CDC14A, Encoding a Protein Phosphatase Involved in Hair Cell Ciliogenesis, Cause Autosomal-Recessive Severe to Profound Deafness. Am J Hum Genet. 2016; 98: 1266–1270. https://doi.org/10.1016/j.ajhg.2016.04.015 PMID: 27259055

28. Li P, He Y, Cai G, Xiao F, Yang J, Li Q, et al. CCDC114 is mutated in patient with a complex phenotype combining primary ciliary dyskinesia, sensorineural deafness, and renal disease. J Hum Genet. 2018; 64: 39–48. https://doi.org/10.1038/s10038-018-0514-z PMID: 30291279

29. Imtiaz A, Belyantseva IA, Beirl AJ, Fenollar-Ferrer C, Bashir R, Bukhari I, et al. CDC14A phosphatase is essential for hearing and male fertility in mouse and human. Hum Mol Genet. 2018; 27: 780–798. https://doi.org/10.1093/hmg/ddx440 PMID: 29293958

30. Kindt KS, Finch G, Nicolson T. Kinocilia mediate mechanosensitivity in developing zebrafish hair cells. Dev Cell. 2012; 23: 329–341. https://doi.org/10.1016/j.devcel.2012.05.022 PMID: 22898777

31. Tsujikawa M, Malicki J. Intraflagellar Transport Genes Are Essential for Differentiation and Survival of Vertebrate Sensory Neurons. Neuron. 2004; 42: 703–716. https://doi.org/10.1016/s0896-6273(04)00268-5 PMID: 15182712

32. Stawicki TM, Hernandez L, Esterberg R, Linbo T, Owens KN, Shah AN, et al. Cilia-associated genes play differing roles in aminoglycoside-induced hair cell death in zebrafish. G3 Genes, Genomes, Genet. 2016; 6: 2225–2235. https://doi.org/10.1534/g3.116.030080 PMID: 27207957

33. Stawicki TM, Linbo T, Hernandez L, Parkinson L, Bellefeuille D, Rubel EW, et al. The role of retrograde intraflagellar transport genes in aminoglycoside-induced hair cell death. Biol Open. 2018. https://doi.org/10.1242/bio.038745 PMID: 30578252

34. Owens KN, Santos F, Roberts B, Linbo T, Coffin AB, Knisely AJ, et al. Identification of genetic and chemical modulators of zebrafish mechanosensory hair cell death. PLoS Genet. 2008; 4: e1000020. https://doi.org/10.1371/journal.pgen.1000020 PMID: 18454195

35. Sedykh I, TeSlaa JJ, Tatarsky RL, Keller AN, Toops KA, Lakkaraju A, et al. Novel roles for the radial spoke head protein 9 in neural and neurosensory cilia. Sci Rep. 2016; 6: 34437. https://doi.org/10.1038/srep34437 PMID: 27687975

36. Nesmith JE, Hostelley TL, Leitch CC, Matern MS, Sethna S, McFarland R, et al. Genomic knockout of alms1 in zebrafish recapitulates Alström syndrome and provides insight into metabolic phenotypes. Hum Mol Genet. 2019; 28: 2212–2223. https://doi.org/10.1093/hmg/ddz053 PMID: 31220269

37. Collin GB, Marshall JD, Ikeda A, So WV, Russell-Eggitt I, Maffei P, et al. Mutations in ALMS1 cause obesity, type 2 diabetes and neurosensory degeneration in Alström syndrome. Nat Genet. 2002; 31: 74–78. https://doi.org/10.1038/ng867 PMID: 11941369

38. Hearn T, Renforth GL, Spalluto C, Hanley NA, Piper K, Brickwood S, et al. Mutation of ALMS1, a large gene with a tandem repeat encoding 47 amino acids, causes Alström syndrome. Nat Genet. 2002; 31: 79–83. https://doi.org/10.1038/ng874 PMID: 11941370

39. Marshall JD, Bronson RT, Collin GB, Nordstrom AD, Maffei P, Paisey RB, et al. New Alström syndrome phenotypes based on the evaluation of 182 cases. Archives of Internal Medicine. American Medical Association; 2005. pp. 675–683. https://doi.org/10.1001/archinte.165.6.675 PMID: 15795345

40. Hearn T, Spalluto C, Phillips VJ, Renforth GL, Copin N, Hanley NA, et al. Subcellular localization of ALMS1 supports involvement of centrosome and basal body dysfunction in the pathogenesis of obesity, insulin resistance, and type 2 diabetes. Diabetes. 2005; 54: 1581–1587. https://doi.org/10.2337/diabetes.54.5.1581 PMID: 15855349

41. Marshall JD, Ludman MD, Shea SE, Salisbury SR, Willi SM, LaRoche RG, et al. Genealogy, natural history, and phenotype of Alström Syndrome in a large Acadian kindred and three additional families. American Journal of Medical Genetics. Wiley-Liss Inc.; 1997. pp. 150–161. https://doi.org/10.1002/(sici)1096-8628(19971212)73:2<150::aid-ajmg9>3.0.co;2-y

42. Lindsey S, Brewer C, Stakhovskaya O, Kim HJ, Zalewski C, Bryant J, et al. Auditory and otologic profile of Alström syndrome: Comprehensive single center data on 38 patients. Am J Med Genet Part A. 2017; 173: 2210–2218. https://doi.org/10.1002/ajmg.a.38316 PMID: 28573831

43. Bahmad F, Costa CSA, Teixeira MS, de Barros Filho J, Viana LM, Marshall J. Familial Alström syndrome: a rare cause of bilateral progressive hearing loss. Braz J Otorhinolaryngol. 2014; 80: 99–104. https://doi.org/10.5935/1808-8694.20140023 PMID: 24830966

44. Nadol JB, Marshall JD, Bronson RT. Histopathology of the human inner ear in Alström's syndrome. Audiol Neurotol. 2015; 20: 267–272. https://doi.org/10.1159/000381935 PMID: 26111748

45. Arsov T, Silva DG, O'Bryan MK, Sainsbury A, Lee NJ, Kennedy C, et al. Fat aussie—A new Alström syndrome mouse showing a critical role for ALMS1 in obesity, diabetes, and spermatogenesis. Mol Endocrinol. 2006; 20: 1610–1622. https://doi.org/10.1210/me.2005-0494 PMID: 16513793

46. Collin GB, Cyr E, Bronson R, Marshall JD, Gifford EJ, Hicks W, et al. Alms1-disrupted mice recapitulate human Alströ m syndrome. Hum Mol Genet. 2005; 14: 2323–2333. https://doi.org/10.1093/hmg/ddi235 PMID: 16000322

47. Jagger D, Collin G, Kelly J, Towers E, Nevill G, Longo-Guess C, et al. Alström Syndrome protein ALMS1 localizes to basal bodies of cochlear hair cells and regulates cilium-dependent planar cell polarity. Hum Mol Genet. 2011; 20: 466–481. https://doi.org/10.1093/hmg/ddq493 PMID: 21071598

48. Stawicki TM, Owens KN, Linbo T, Reinhart KE, Rubel EW, Raible DW. The zebrafish merovingian mutant reveals a role for pH regulation in hair cell toxicity and function. Dis Model Mech. 2014; 7: 847–856. https://doi.org/10.1242/dmm.016576 PMID: 24973752

49. Koleilat A, Dugdale JA, Christenson TA, Bellah JL, Lambert AM, Masino MA, et al. L-type voltage-gated calcium channel agonists mitigate hearing loss and modify ribbon synapse morphology in the zebrafish model of Usher syndrome type 1. Dis Model Mech. 2020; 13: dmm043885. https://doi.org/10.1242/dmm.043885 PMID: 33361086

50. Raible DW, Kruse GJ. Organization of the lateral line system in embryonic zebrafish. J Comp Neurol. 2000; 421: 189–198. PMID: 10813781

51. Collin GB, Marshall JD, King BL, Milan G, Maffei P, Jagger DJ, et al. The alström syndrome protein, ALMS1, interacts with α-actinin and components of the endosome recycling pathway. PLoS One. 2012; 7: e37925. https://doi.org/10.1371/journal.pone.0037925 PMID: 22693585

52. Chen JH, Geberhiwot T, Barrett TG, Paisey R, Semple RK. Refining genotype–phenotype correlation in Alström syndrome through study of primary human fibroblasts. Mol Genet Genomic Med. 2017; 5: 390–404. https://doi.org/10.1002/mgg3.296 PMID: 28717663

53. Li G, Vega R, Nelms K, Gekakis N, Goodnow C, McNamara P, et al. A role for Alström syndrome protein, Alms1, in kidney ciliogenesis and cellular quiescence. PLoS Genet. 2007; 3: e8. https://doi.org/10.1371/journal.pgen.0030008 PMID: 17206865

54. Graser S, Stierhof YD, Lavoie SB, Gassner OS, Lamla S, Le Clech M, et al. Cep164, a novel centriole appendage protein required for primary cilium formation. J Cell Biol. 2007; 179: 321–330. https://doi.org/10.1083/jcb.200707181 PMID: 17954613

55. Gale JE, Marcotti W, Kennedy HJ, Kros CJ, Richardson GP. FM1-43 dye behaves as a permeant blocker of the hair-cell mechanotransducer channel. J Neurosci. 2001; 21: 7013–7025. https://doi.org/10.1523/JNEUROSCI.21-18-07013.2001 PMID: 11549711

56. Meyers JR, MacDonald RB, Duggan A, Lenzi D, Standaert DG, Corwin JT, et al. Lighting up the senses: FM1-43 loading of sensory cells through nonselective ion channels. J Neurosci. 2003; 23: 4054–4065. https://doi.org/10.1523/JNEUROSCI.23-10-04054.2003 PMID: 12764092

57. Seiler C, Nicolson T. Defective calmodulin-dependent rapid apical endocytosis in zebrafish sensory hair cell mutants. J Neurobiol. 1999; 41: 424–434. PMID: 10526320

58. Huang P, Schier AF. Dampened Hedgehog signaling but normal Wnt signaling in zebrafish without cilia. Development. 2009; 136: 3089–98. https://doi.org/10.1242/dev.041343 PMID: 19700616

59. Duldulao NA, Lee S, Sun Z. Cilia localization is essential for in vivo functions of the Joubert syndrome protein Arl13b/Scorpion. Development. 2009; 136: 4033–42. https://doi.org/10.1242/dev.036350 PMID: 19906870

60. Cao Y, Park A, Sun Z. Intraflagellar transport proteins are essential for cilia formation and for planar cell polarity. J Am Soc Nephrol. 2010; 21: 1326–33. https://doi.org/10.1681/ASN.2009091001 PMID: 20576807

61. Michaud JL, Heon E, Guilbert F, Weill J, Puech B, Benson L, et al. Natural history of Alstrom syndrome in early childhood: Onset with dilated cardiomyopathy. J Pediatr. 1996; 128: 225–229. https://doi.org/10.1016/s0022-3476(96)70394-3 PMID: 8636816

62. Nicolson T. Ribbon synapses in zebrafish hair cells. Hearing Research. Elsevier B.V.; 2015. pp. 170–177. https://doi.org/10.1016/j.heares.2015.04.003 PMID: 25916266

63. Nicolson T. The genetics of hair-cell function in zebrafish. J Neurogenet. 2017; 31: 102–112. https://doi.org/10.1080/01677063.2017.1342246 PMID: 28705044

64. Barta CL, Liu H, Chen L, Giffen KP, Li Y, Kramer KL, et al. RNA-seq transcriptomic analysis of adult zebrafish inner ear hair cells. Sci Data. 2018; 5: 180005. https://doi.org/10.1038/sdata.2018.5 PMID: 29406519

65. Erickson T, Nicolson T. Identification of sensory hair-cell transcripts by thiouracil-tagging in zebrafish. BMC Genomics. 2015; 16: 842. https://doi.org/10.1186/s12864-015-2072-5 PMID: 26494580

66. Lush ME, Diaz DC, Koenecke N, Baek S, Boldt H, St Peter MK, et al. scRNA-Seq reveals distinct stem cell populations that drive hair cell regeneration after loss of Fgf and Notch signaling. Elife. 2019; 8. https://doi.org/10.7554/eLife.44431 PMID: 30681411

67. Rossi A, Kontarakis Z, Gerri C, Nolte H, Hölper S, Krüger M, et al. Genetic compensation induced by deleterious mutations but not gene knockdowns. Nature. 2015; 524: 230–233. https://doi.org/10.1038/nature14580 PMID: 26168398

68. Knorz VJ, Spalluto C, Lessard M, Purvis TL, Adigun FF, Collin GB, et al. Centriolar association of ALMS1 and likely centrosomal functions of the ALMS motif-containing proteins C10orf90 and KIAA1731. Mol Biol Cell. 2010; 21: 3617–3629. https://doi.org/10.1091/mbc.E10-03-0246 PMID: 20844083

69. Hearn T. ALMS1 and Alström syndrome: a recessive form of metabolic, neurosensory and cardiac deficits. J Mol Med. 2019; 97: 1–17. https://doi.org/10.1007/s00109-018-1714-x PMID: 30421101

70. Taylor JS, Braasch I, Frickey T, Meyer A, Van de Peer Y. Genome duplication, a trait shared by 22,000 species of ray-finned fish. Genome Res. 2003; 13: 382–390. https://doi.org/10.1101/gr.640303 PMID: 12618368

71. Prince VE, Pickett FB. Splitting pairs: The diverging fates of duplicated genes. Nature Reviews Genetics. Nat Rev Genet; 2002. pp. 827–837. https://doi.org/10.1038/nrg928 PMID: 12415313

72. Erickson T, Pacentine I V., Venuto A, Clemens R, Nicolson T. The lhfpl5 Ohnologs lhfpl5a and lhfpl5b Are Required for Mechanotransduction in Distinct Populations of Sensory Hair Cells in Zebrafish. Front Mol Neurosci. 2020; 12: 320. https://doi.org/10.3389/fnmol.2019.00320 PMID: 32009898

73. Lv C, Stewart WJ, Akanyeti O, Frederick C, Zhu J, Santos-Sacchi J, et al. Synaptic Ribbons Require Ribeye for Electron Density, Proper Synaptic Localization, and Recruitment of Calcium Channels. Cell Rep. 2016; 15: 2784–2795. https://doi.org/10.1016/j.celrep.2016.05.045 PMID: 27292637

74. Chou S-W, Chen Z, Zhu S, Davis RW, Hu J, Liu L, et al. A molecular basis for water motion detection by the mechanosensory lateral line of zebrafish. Nat Commun. 2017; 8: 2234. https://doi.org/10.1038/s41467-017-01604-2 PMID: 29269857

75. Singh PP, Isambert H. OHNOLOGS v2: A comprehensive resource for the genes retained from whole genome duplication in vertebrates. Nucleic Acids Res. 2020; 48: D724–D730. https://doi.org/10.1093/nar/gkz909 PMID: 31612943

76. Burns JC, Kelly MC, Hoa M, Morell RJ, Kelley MW. Single-cell RNA-Seq resolves cellular complexity in sensory organs from the neonatal inner ear. Nat Commun. 2015; 6: 8557. https://doi.org/10.1038/ncomms9557 PMID: 26469390

77. Scheffer DI, Shen J, Corey DP, Chen ZY. Gene expression by mouse inner ear hair cells during development. J Neurosci. 2015; 35: 6366–6380. https://doi.org/10.1523/JNEUROSCI.5126-14.2015 PMID: 25904789

78. May-Simera HL, Ross A, Rix S, Forge A, Beales PL, Jagger DJ. Patterns of expression of Bardet-Biedl syndrome proteins in the mammalian cochlea suggest noncentrosomal functions. J Comp Neurol. 2009; 514: 174–88. https://doi.org/10.1002/cne.22001 PMID: 19396898

79. Jones C, Roper VC, Foucher I, Qian D, Banizs B, Petit C, et al. Ciliary proteins link basal body polarization to planar cell polarity regulation. Nat Genet. 2008; 40: 69–77. https://doi.org/10.1038/ng.2007.54 PMID: 18066062

80. Sipe CW, Lu X. Kif3a regulates planar polarization of auditory hair cells through both ciliary and non-ciliary mechanisms. Development. 2011; 138: 3441–3449. https://doi.org/10.1242/dev.065961 PMID: 21752934

81. Lim DJ, Anniko M. Developmental morphology of the mouse inner ear. A scanning electron microscopic observation. Acta Otolaryngol Suppl. 1985; 422: 1–69. PMID: 3877398