Loss of Otoferlin alters the transcriptome profile and ribbon synapse architecture of sensory hair cells

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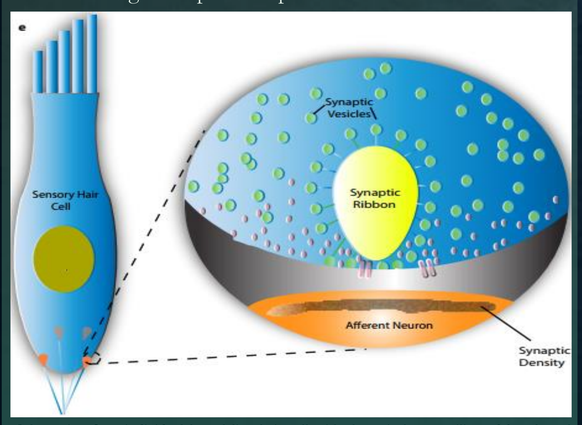
ABSTRACT

Otoferlin is an essential vesicular membrane protein present in hair cells, that facilitates synaptic vesicle trafficking and has been linked to nonsyndromic hearing loss in humans. While it is required for hearing, the effect of its loss on hair cell and the hearing pathway are still unknown. We found that in addition to reducing synaptic vesicle recycling, loss of otoferlin also leads to mistrafficking of synaptic vesicle proteins and larger but fewer synaptic ribbons, indicating that otoferlin contributes to ribbon synapse morphology. Loss of otoferlin altered the expression levels of genes that are involved in calcium handling, as well as those that are important for neural development and morphogenesis of nearby neurons. This leads us to the conclusion that otoferlin has a much more complex functional pathway, and it is involved in an array of different roles within the hair cell.

INTRODUCTION

Auditory and vestibular sensory hair cells transform mechanical forces into chemical signals for the purposes of hearing and balance. Hair cells are known to house a protein, otoferlin that has been linked to a form of nonsyndromic hearing loss[1]. Otoferlin binds calcium[2], and deletion of otoferlin results in a loss of neurotransmitter release[3], suggesting a role in calcium sensitive synaptic vesicle priming and fusion[4-6]. Unlike other synaptic proteins , otoferlin does not traffic exclusively to the ribbon synapse region but rather is distributed broadly in the cell body were it interacts with non-synaptic proteins[7-9]. It remains to be determined however, whether loss of otoferlin significantly alters processes beyond synaptic vesicle exocytosis.

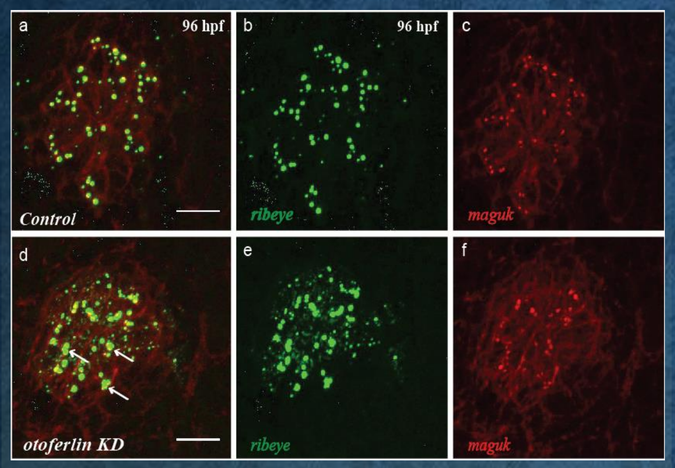
We find that in addition to the well characterized reduction in synaptic vesicle recycling, loss of otoferlin also results in mistrafficking of synaptic vesicle proteins and larger but fewer synaptic ribbons. In addition, analysis of the transcriptome profile of zebrafish lacking otoferlin revealed altered expression levels of many genes associated with calcium handling, amino acid transport, axonal guidance, and neuronal growth. We conclude that loss of otoferlin both disrupts ribbon synapse architecture and disrupts the homeostatic gene expression profile of the hair cell.



Schematic of an individual lateral line hair cell with the apical stereocilia and basolateral afferent neurons. Dotted black circle indicates the area between the sensory hair cell pre (blue) and post synapse (orange). A closer look at the cross-section of the dotted area shows the molecular structure of the active zone of a hair cell synapse as indicated by the presence of a presynaptic ribbon body (yellow), synaptic vesicles (green), calcium channels and calcium ions at the active zone (purple) and the post-synaptic density (brown).

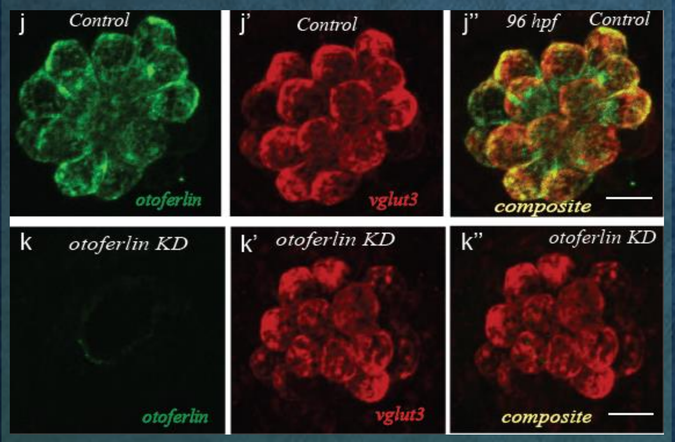
RESULTS

1. Loss of otoferlin expression results in fewer but larger ribbon synapses.



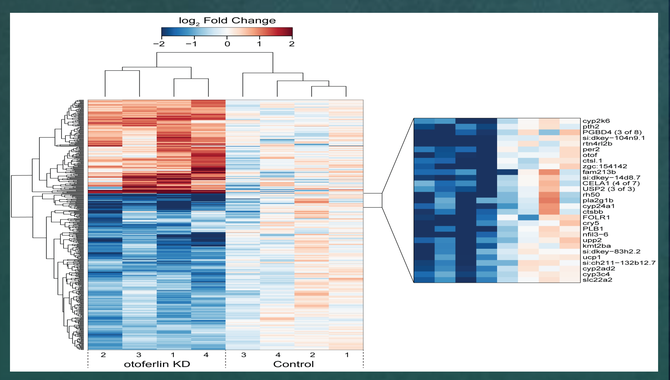
Confocal images showing distribution of Ribeye and MAGUK (yellow) (a), Ribeye (green) (b) and MAGUK (red) (c) in control neuromast hair cells, (d-f) Confocal images of Ribeye and MAGUK distribution in otoferlin KD (d), Ribeye (e), and MAGUK (f) distributions in otoferlin depleted neuromasts

2. Loss of otoferlin expression results in mistrafficking of Vglut3



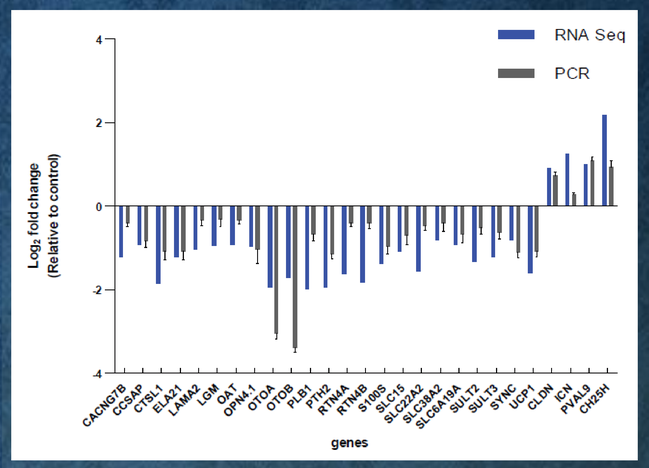
(j-j") VGlut3 distribution in control, and (k-k") otoferlin depleted larvae. Representative confocal z-projections of otoferlin (green), VGlut3 (red) and composite (yellow) immunolabel in control and otoferlin depleted neuromast hair cells

3. Otoferlin depletion alters the transcriptional profile in zebrafish



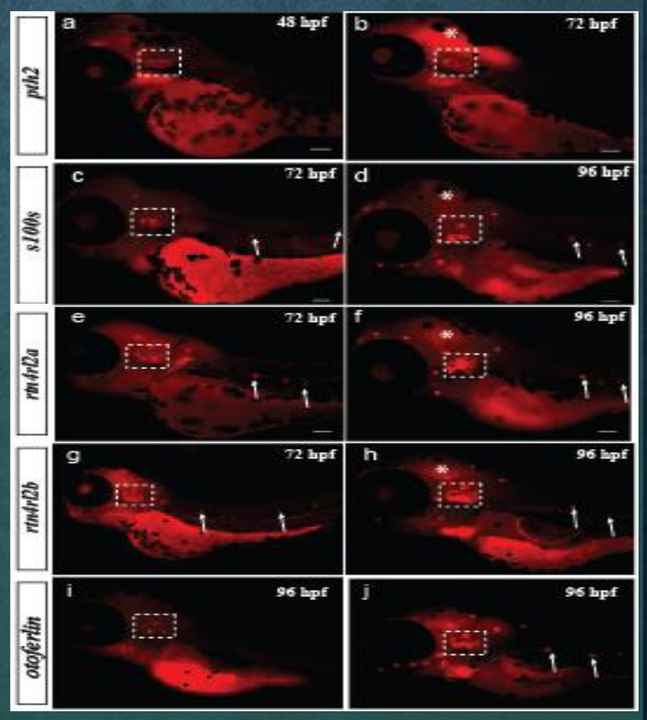
Bi-hierarchically clustered heatmap of the 433 significantly differentially expressed transcripts in otoferlin depleted larvae (FDR adjusted P-value ≤ 0.05; fold change ≥ 1.5). The inset on the right highlights the cluster of the most highly decreased transcripts.

4. Transcriptomics gene expression profiles validated by qPCR



Comparison of RNA sequencing and qPCR for differentially expressed genes in otoferlin depleted larvae. **Black bar graphs** represents relative mRNA expression changes of the upregulated and downregulated genes derived from transcriptomic analysis (no. of biological reps. = 4, each replicate consists of 15 pooled embryos). **Brown bars** represent qPCR data of mRNA expression changes of genes in larval otoferlin mutant zebrafish at 96 hpf

5. Temporal and spatial expression of some of qPCR validated transcripts in the lateral line neuromast and otic region using in situ hybridization



Expressions of pth2 were found primarily in the otic region at 48 hpf and 72 hpf and also in the brain at 72 hpf (Figs. 6a-b). Expression in the lateral line was not detected39. By contrast, expressions of s100s were detected in both neuromasts of the lateral line and otic regions at 72 hpf and 96 hpf (Figs. 6c-d). Transient s100s expressions were also detected in the brain at 96 hpf. Figs. 6e-f showed localization of rtn4rl2a transcripts in the lateral line neuromasts and otic placode at 72 hpf and 96 hpf, with expression in the otic placode, which became predominant at 96 hpf (Fig. 6f) corresponding with maturation of the sensory cells in the otic region14. Rtn4rl2b transcripts were detected in the lateral line neuromasts and otic region at 72 hpf and 96 hpf, with expression in the otic placode becoming pronounced at 96 hpf (Figs. 6g-h) corresponding with maturation of the sensory cells14. The expression of all these transcripts pattern bore a high degree of resemblance with expression of otoferlin in the lateral line neuromast and otic region (Fig. 6i-j).

CONCLUSION

1. Otoferlin depleted hair cells have fewer but larger synaptic ribbons as well as mistrafficked VGlut3.
2. Analysis of the RNA-Seq data revealed that loss of otoferlin results in lower levels of the EF-hand proteins parvalbumin 3 and s100s, which act as calcium homeostasis and buffering proteins.
3. Parathyroid hormone pth2, which is also associated with calcium signalling, was downregulated in otoferlin depleted hair cells.
4. We speculate that the reduced number of transcripts in neuromasts may represent an attempt to compensate for loss of signalling between the hair cell and afferent neuron.

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