A Study of PDZ Domain-Containing 7 (PDZD7) in the Mouse Retina

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Purpose

PDZD7 is a newly identified modifier and contributor gene of Usher syndrome (USH). In the inner ear, PDZD7 colocalizes with GPR98, an USH2C protein, at ankle links in cochlear and vestibular hair cells. Therefore, PDZD7 is proposed to be a novel component of the USH2 complex, which is composed of the three known USH2 causative proteins. In this study, we investigated PDZD7 expression and its role in the organization of the USH2 complex in the retina.

Methods

The expression of Pdzd7 was examined at the mRNA and protein levels using RT-PCR, western blotting, and immunostaining assays. A Pdzd7 knockout mouse was generated by gene trapping and characterized phenotypically by immunostaining and electroretinogram.

Results

1. *Pdzd7* knockout mice were generated.

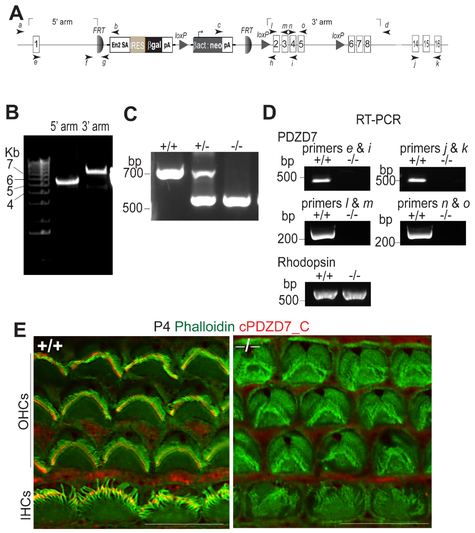

Fig. 1. (A) A schematic diagram of the gene targeting Pdzd7$^{tm1a(EUCOMM)Wtsi}$ allele. (B) Confirmation of insertion of the targeting cassette into the Pdzd7 gene by PCR using primer pairs of a/b and c/d. (C) Identification of the mutant allele by routine genotyping PCR using primers, f, h, and g. (D) Disrupted transcription of the Pdzd7 gene shown by RT-PCR analysis in the retina using primers e, i, j, k, l, m, n and o. RT-PCR of rhodopsin was used here as a positive control. Note, primer pairs of l/m, n/o and j/k were designed to amplify PDZ1, PDZ2 and PDZ3 regions of PDZD7 cDNA, respectively. (E) Immunofluorescence demonstrates loss of PDZD7 expression in Pdzd7$^{-/-}$ cochlear hair cells at P4. Scale bars, 10 μm. cPDZD7_C, chicken antibody against PDZD7_C; +/+, wild-type; -/-, Pdzd7 knockout.

2. Several N-terminal but not full-length PDZD7 mRNA splice variants were found in the adult mouse retina.

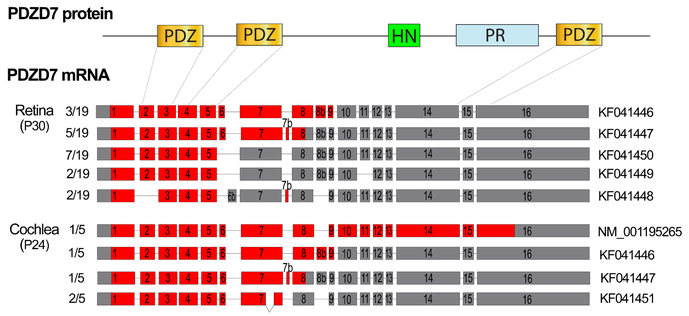

Fig. 2. Top, domain structure of the full-length PDZD7 protein. Bottom, Pdzd7 splice variants found in the P30 retina and P24 cochlea. Exons encoding the PDZ domains are indicated by dashed lines. Red regions are protein encoding regions. Grey regions are 5' or 3' untranslated regions. The numbers on the left of each splice variant are the count of the splice variant (before the slash) and the total number of splice variants screened (after the slash). The numbers on the right of splice variants are their GenBank accession numbers.

3. Full-length PDZD7 is expressed in the developing but not adult mouse retina.

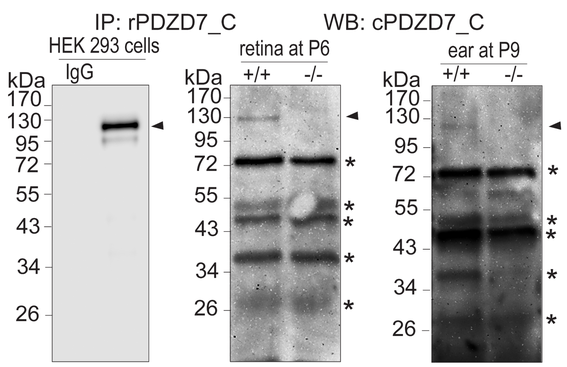

Fig. 3. Western blotting (WB) following immunoprecipitation (IP) found full-length PDZD7 protein expression in the retina at P6 (middle) and in the cochlea at P9 (right) in wild-type mice. Pdzd7$^{-/-}$ mice and HEK293 cells transfected with full-length PDZD7 were used as negative and positive control, respectively. The same experiment using adult wild-type mouse retinas could not detect the protein expression of full-length PDZD7 and N-terminal PDZD7 isoforms. Arrowheads point to the PDZD7 bands. Asterisks mark non-specific bands. IgG, non-immune rabbit immunoglobulin; rPDZD7_C, rabbit antibody against PDZD7_C ; cPDZD7_C, chicken antibody against PDZD7_C.

4. Ablation of PDZD7 does not affect the localization of the three USH2 proteins in the retina.

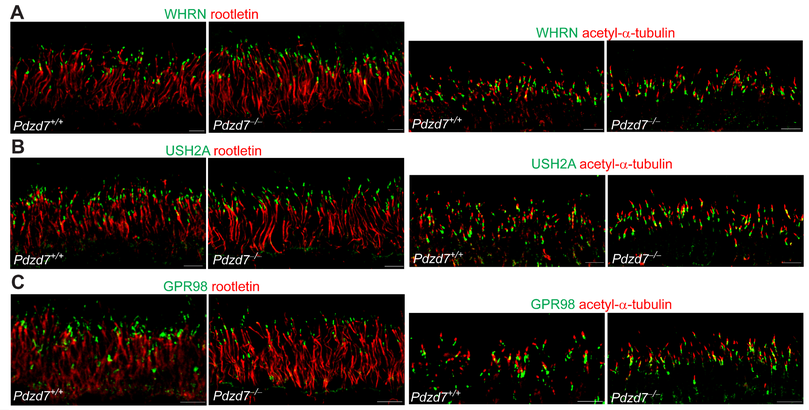

Fig. 4. Compared to wild-type (Pdzd7$^{+/+}$) photoreceptors, the distributions of WHRN (green, A), USH2A (green, B), and GPR98 (green, C), relative to the ciliary rootlet (rootletin, red, left panels) and the axonemal microtubules (acetylated α-tubulin, red, right panels), appeared normal in Pdzd7$^{-/-}$ photoreceptors. Similar results were obtained from three pairs of Pdzd7$^{+/+}$ and Pdzd7$^{-/-}$ mice. Scale bars, 5 μm.

5. *Pdzd7* knockout mice appear to have normal ERGs at about one month of age.

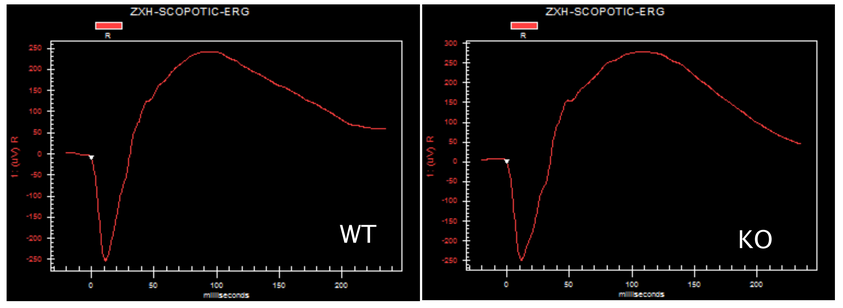

Fig. 5. Representative scotopic ERG tracings from wild-type and Pdzd7 knockout mice at 40 days of age. Light intensity is 0.48 lg cds/m^2.

Conclusions

Despite the existence of multiple splice variants, PDZD7 expression at the protein level is very low in the retina. PDZD7 is not as important as WHRN in organizing the USH2 complex in photoreceptors.

Acknowledgments

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