
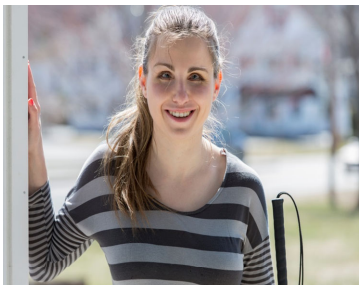


CRISPR gene editing rescues deficits in human *USH2A* mutant retinal organoids

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Editas Medicine, Inc., Cambridge, MA, USA

ARVO Annual Meeting 2021

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Disclosures

- Employees and shareholders of Editas Medicine: N.D., R.P., S.J.
- Former employees of Editas Medicine: N.H., B.D., C.A.

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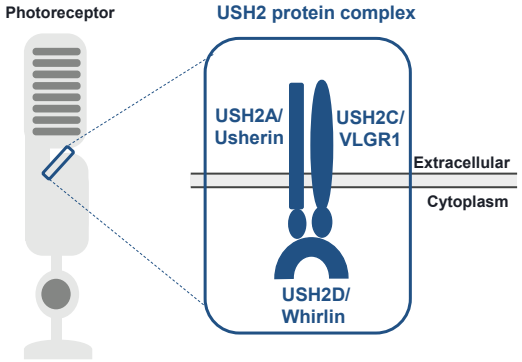
Introduction

Background

- USH2A, a retinal degenerative disease leading to hearing and vision loss, is most commonly caused by recessive mutations in exon 13 of the *USH2A* gene encoding the USH2A protein
- USH2A protein is localized in the connecting cilium between inner and outer segments of photoreceptors, and supports inner/outer segment formation and photoreceptor morphology

USH2 protein complex



USH2A, Usher syndrome type II

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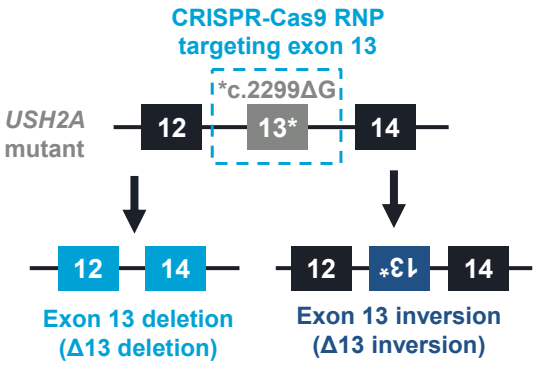
Objective

Objective

- To investigate whether deleting or inverting *USH2A* exon 13 could restore deficits caused by mutations at this site in a novel induced pluripotent stem cell (iPSC)-derived retinal organoid model

CRISPR-Cas9 editing of *USH2A* exon 13



RNP, ribonucleotide protein

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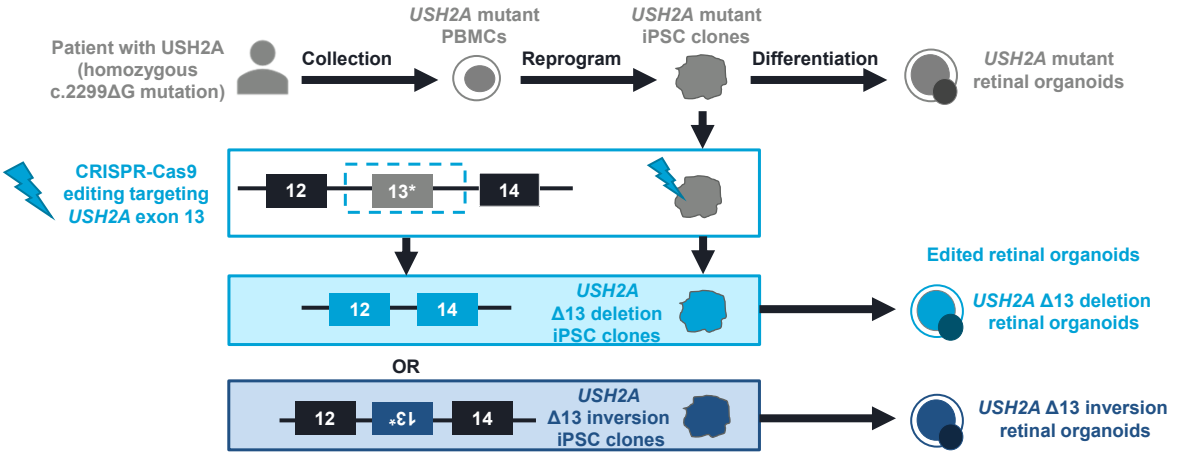
Methods



Retinal organoids were characterized at various timepoints during organogenesis by gene expression (RT-qPCR), IHC, and morphology

*c.2299ΔG homozygous mutation
IHC, immunohistochemistry; PBMC, peripheral blood mononuclear cell; RT-qPCR, real-time quantitative polymerase chain reaction

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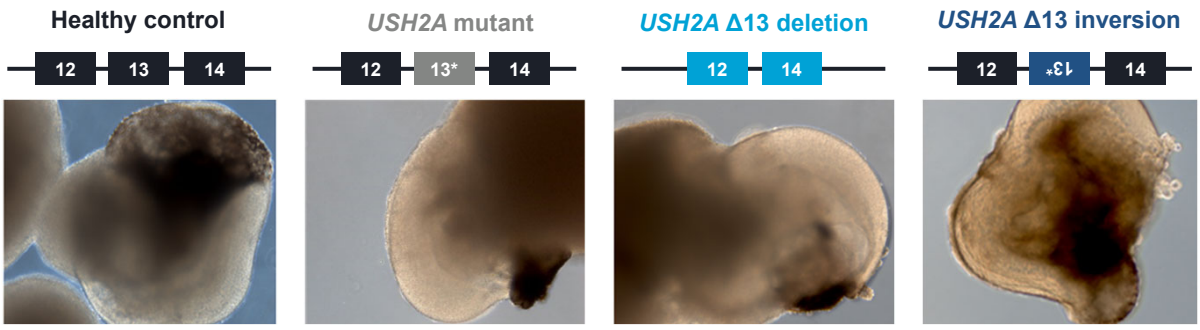
Edited iPSC clones successfully differentiated to retinal organoids

10X images at Day 100



Retinal organoids derived from healthy, unedited, and edited *USH2A* iPSCs had similar morphology at Day 100

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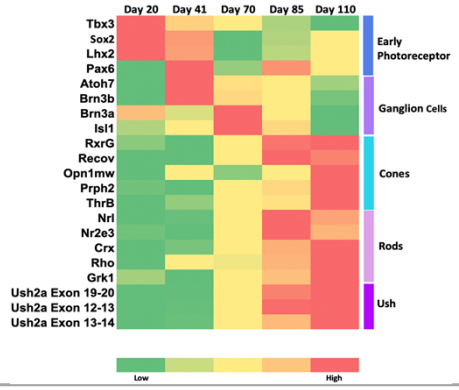
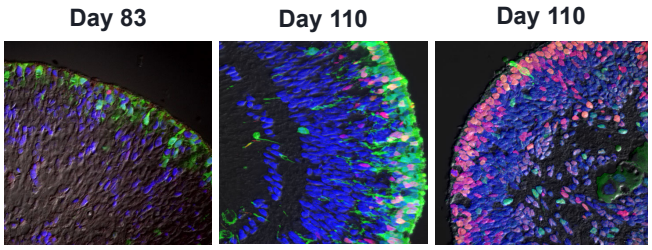
Retinal organoids derived from iPSCs demonstrated time-dependent expression of developmental genes

Developmental gene expression profile



Expression of retinal protein markers (IHC)



Recoverin
Nrl
DAPI

Recoverin
Nrl
DAPI

L/M opsin
Crx
DAPI

Expression patterns of retinal markers in retinal organoids are consistent with temporal gene regulation during retinal development *in vivo*

Crx, cone rod homeobox (cone-specific transcription factor); DAPI, 4′,6-diamidino-2-phenylindole (nuclei marker); L/M opsin, long- and middle-wavelength sensitive opsin; Nrl, neural retina leucine zipper (rod-specific transcription factor)
Note: different batches of organoids used for RT-qPCR and IHC

Hoshino, et al. Dev Cell 2017
Mellough, et al. Development 2019

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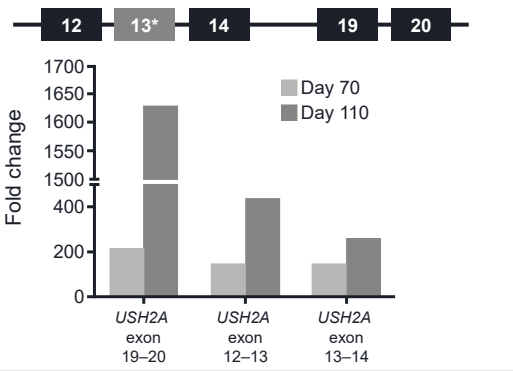
USH2A exon 13 is absent from retinal organoids derived from edited iPSCs

RT-qPCR at Days 70/76 and 110

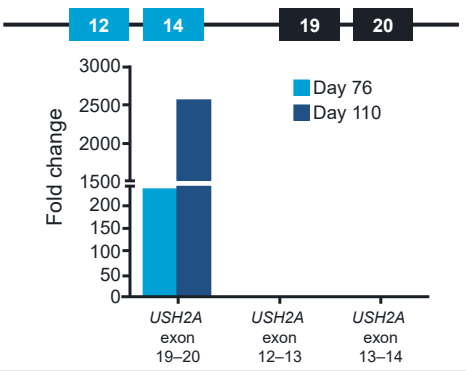
***USH2A* mutant**

P1 P2 P3 (positive control)



***USH2A* Δ13 deletion**

P3 (positive control)



***USH2A* transcripts were detected in retinal organoids from Day 70 and stayed stable after that**

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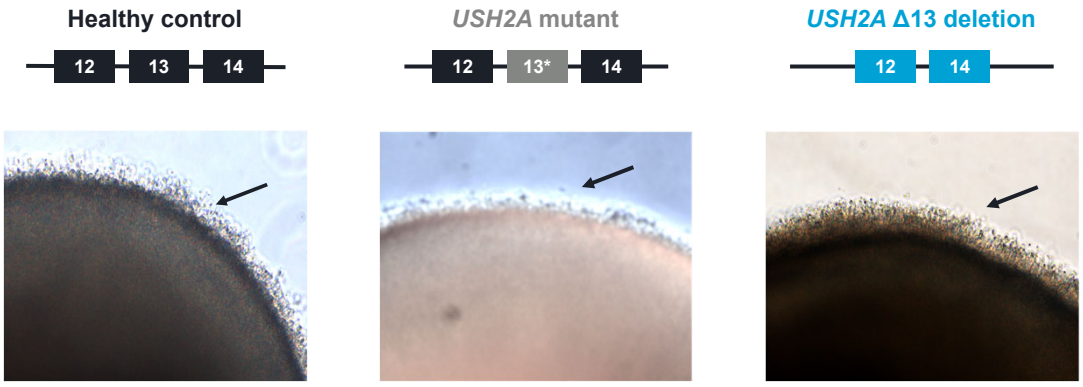
USH2A Δ13 deletion organoids had similar photoreceptor extensions to healthy controls

20X images at Day 215



The mutant organoids had shorter and less dense extensions than healthy control or *USH2A* Δ13 deletion organoids, with disorganized structure

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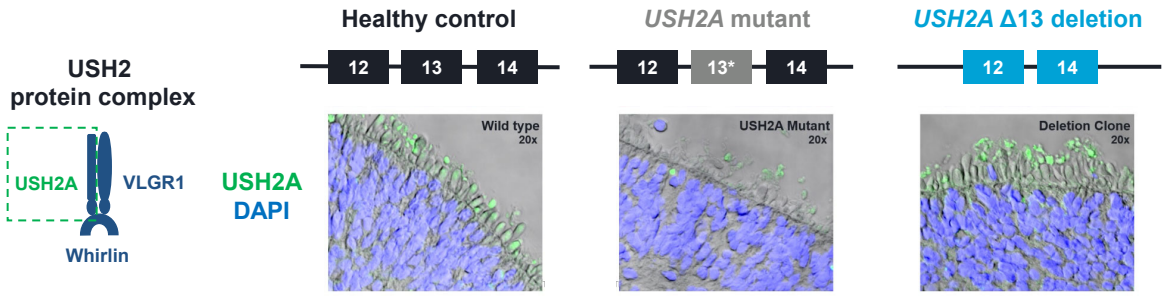
USH2A Δ13 deletion retinal organoids had restored USH2A protein expression

20X images at Day 215



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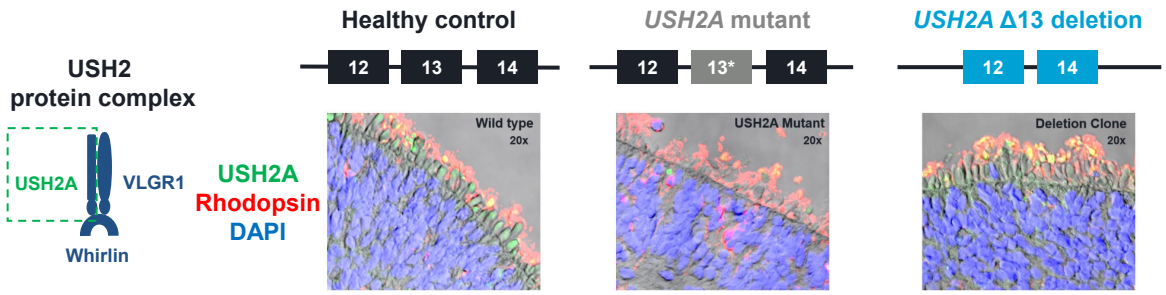
USH2A Δ13 deletion retinal organoids showed highly organized photoreceptor morphology, similar to that of healthy controls

20X images at Day 215



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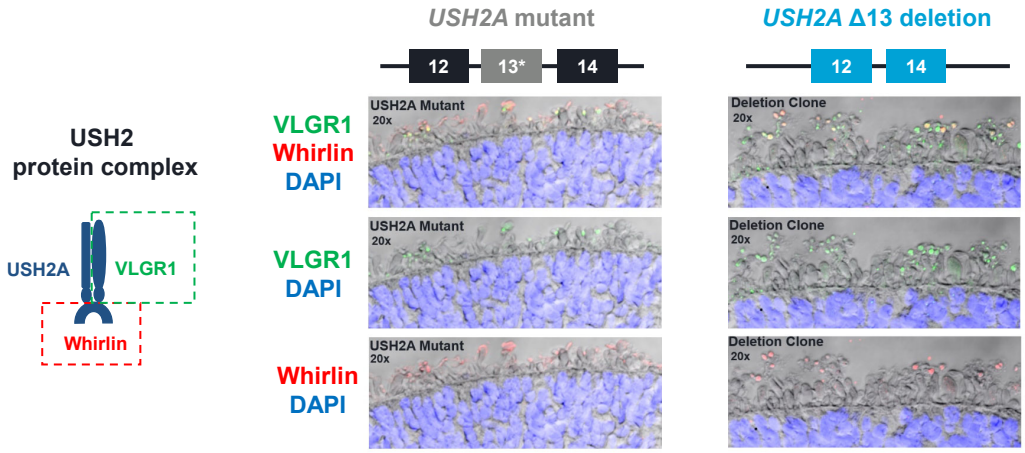
USH2A protein complex, with VLGR1 and whirlin, was restored in *USH2A* Δ13 deletion organoids

20X images at Day 215



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Conclusions

Retinal organoids derived from iPSCs **provide a robust and viable model to study human retinal development** *ex vivo*

This proof-of-concept study demonstrated that CRISPR-edited *USH2A* exon 13 deletion **restored USH2 protein complex expression** and **rescued deficits in photoreceptor morphology** in **human retinal organoids** for the first time

These results support using ***USH2A* exon 13 deletion** as a **therapeutic strategy for the treatment of *USH2A*-associated retinal degenerative diseases**

Acknowledgments

- The authors would like to thank all of their Editas colleagues for helping to plan, perform, analyze, and present this work
- The authors gratefully acknowledge Dr. Eric Pierce and Dr. Qin Liu from the Massachusetts Eye and Ear Institute (MEEI) for providing peripheral blood mononuclear cells from a patient with USH2A, which were used in the research presented here in the form of iPSCs
- Editorial assistance was provided by Hilary Wong, PhD, of 2 the Nth (Cheshire, UK) and was funded by Editas Medicine

