CRISPR Base Editing as a Potential Therapeutic Approach for Kir7.1 Channelopathy

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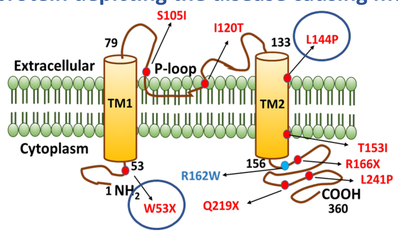
BACKGROUND

- Leber Congenital Amaurosis 16 (LCA16) is a severe form of inherited ocular channelopathy caused by point mutations in *KCNJ13,* which affect the retinal pigment epithelial (RPE).
- AAV-gene therapy related immune responses, CRISPR/Cas9 gene editing associated off-targets, and un-intended indels pose some challenges in clinical use of such treatments.

Kir7.1 protein depicting the disease causing mutations

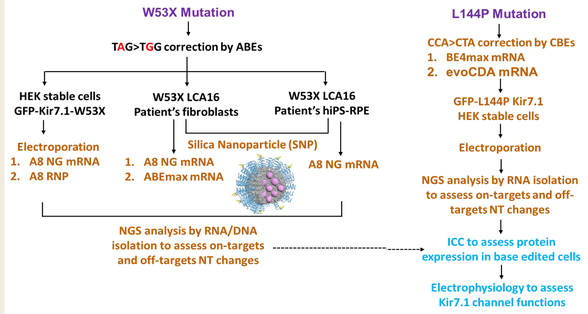


- We used Adenosine and Cytosine CRISPR base editors (ABE and CBE) for proof-of-concept correction of W53X (c.158G>A) and L144P (c.431T> C) in *KCNJ13* using electroporation or silica nanoparticle-mediated delivery to induced pluripotent stem cell derived RPE (iPSC-RPE).

METHODS

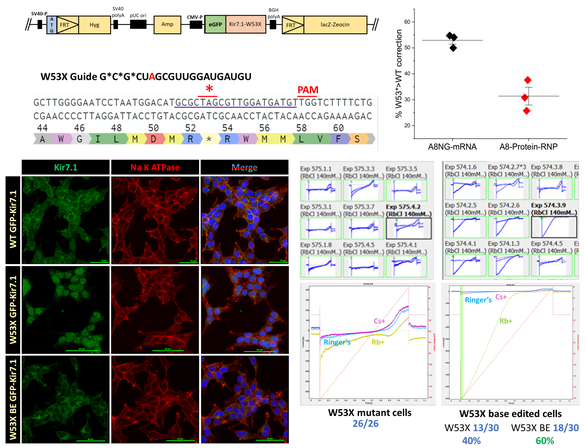
- Base editing was carried out using either ABE or CBE mRNA with a guide RNAs specific to the W53X or L144P mutant allele.
- A HEK293-FRT stable cells (Kir7.1-L144P and Kir7.1- W53X) were base edited via electroporation.
- LCA16-W53X patient-specific fibroblasts and iPS-RPE cells were targeted with ABE mRNA delivered using nanoparticles.
- Base editing efficiency (by deep sequencing), protein expression and localization (Immunocytochemistry), and channel function (electrophysiology) were assessed in edited cells and were compared with non-edited mutant and WT cells. Potential off-targets were screened to evaluate the accuracy and efficacy of CRISPR BEs.

CRISPR ABE and CBE mediated correction in cells

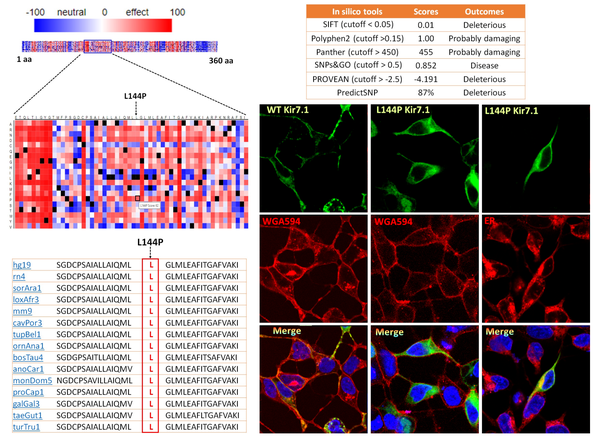


RESULTS

ABE for W53X (TAG>TGG) correction in stable cells

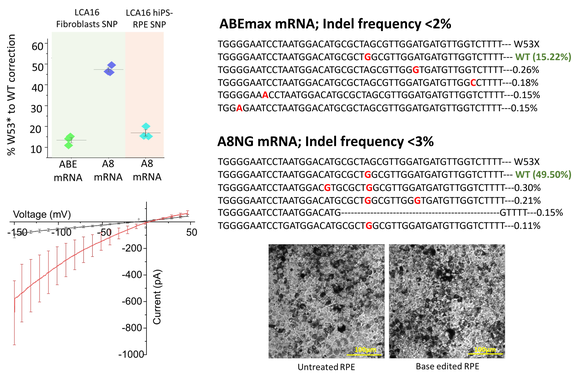


L144P, c.431T>C (CCA>CTA)

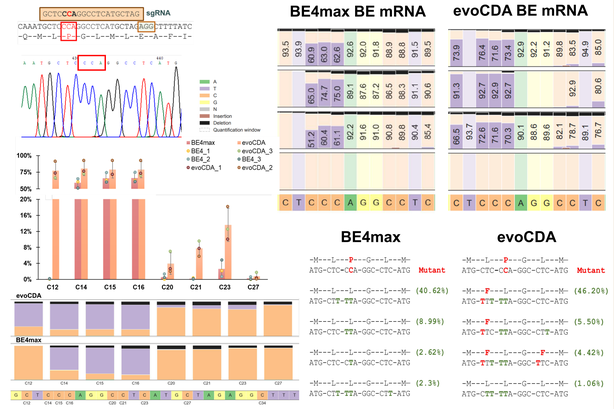


In silico tools	Scores	Outcomes
SIFT (cutoff < 0.05)	0.01	Deleterious
Polyphen2 (cutoff >0.15)	1.00	Probably damaging
Panther (cutoff > 450)	455	Probably damaging
SNPs&GO (cutoff > 0.5)	0.852	Disease
PROVEAN (cutoff > -2.5)	-4.191	Deleterious
PredictSNP	87%	Deleterious

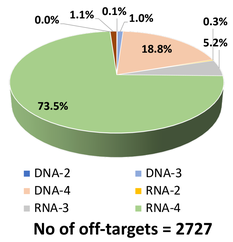
ABE for W53X correction in fibroblasts and hiPS-RPE



CBE for L144P (CCA>CTA) correction in stable cells



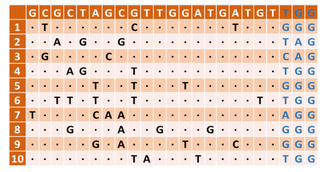
Potential off targets of W53X BE gRNA



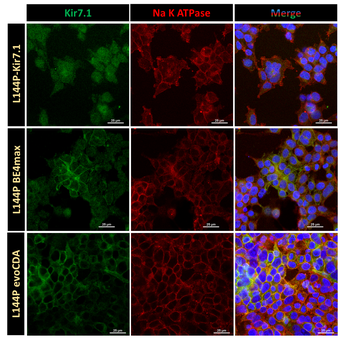
No of off-targets = 2727

<0.01% substitutions and indels for 10 potential sites

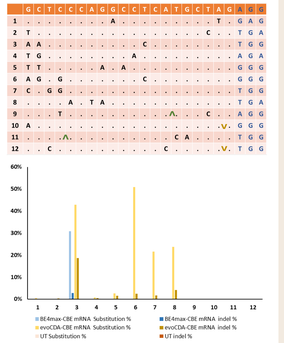
Gene/Region/Location	Chromosome	strand	mismatches
STK24	13	-	3
GRCh38:133058732 Intergenic	10	-	3
GRCh38:50530925 Intergenic	18	-	2
PPM1K Intronic	4	+	3
AP2B1 Intronic	17	-	4
TMEM117 Intronic	12	+	4
MMAA Intronic	4	-	4
GRCh38:113728054 Intronic	4	+	4
HRH1 Intronic	3	-	4
GRCh38:55352815 Intergenic	12	-	3



Protein expression in edited cells



Off-targets of L144P gRNA



MAIN FINDINGS

W53X mutation

- **ABE mRNA (50% editing efficiency) showed higher efficiency than RNP for W53X correction in stable cells (25%).**
- **Nanoparticle-mediated delivery of BEs in fibroblasts (47% editing) and iPSC-RPE (20% editing) established the use of CRISPR BE for *in vivo* BE delivery.**
- **On target indel mutagenesis (<3%) and deep sequencing of potential off-target sites (<0.01%) indicated high accuracy of the ABEs.**
- **Electrophysiology demonstrated robust rescue of channel function in the edited stable and iPSC-RPE cells.**

L144P mutation

- **BE4max mRNA showed better on-target correction efficiency (60%) and fewer off-target effects than evoCDA mRNA.**
- **The codon degeneracy of "Leucine" provides additional flexibility to correct L144P point mutation.**
- **Higher rate of undesired nonsynonymous editing was observed due to multiple bystander 'C' within the editing window.**

CONCLUSIONS

Our results show application of CRISPR base editing for precise correction of point mutations with reduced off-targets compared to CRISPR/Cas9-mediated gene editing. Restoration of channel function in edited iPSC-RPE cells suggests potential of CRISPR BE as a treatment for childhood blindness.

ACKNOWLEDGMENTS

Bikash Pattnaik, PhD
Pawan Shahi, PhD
Allison Spilllane
Katie Beverley
Yu Chang
Enes Akyuz, PhD

David Gamm, MD, PhD
Divya Sinha, PhD
Kim Edwards

Mariya Moosajee, MBBS, PhD

Kris Saha, PhD
Amr Abdeen, PhD
Shivani Saxena
Kaivalya Molugu

David R. Liu, PhD
Greg Newby, PhD

Sarah Gong, PhD
Yuyuan Wang, PhD

Sophion Bioscience

Funding support

Stem Cell & Regenerative Medicine Center SCRMC Fellowship

McPherson Eye Research Institute

NIH National Eye Institute
R24EY032434
R01EY024995

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