Developing Prime Editors to Treat Repeat Expansion Diseases

AIChE CRISPR Technologies
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Prime Medicine, Cambridge MA

Prime Editing: a gene editing technology that is programmable for both *search* and *replace*

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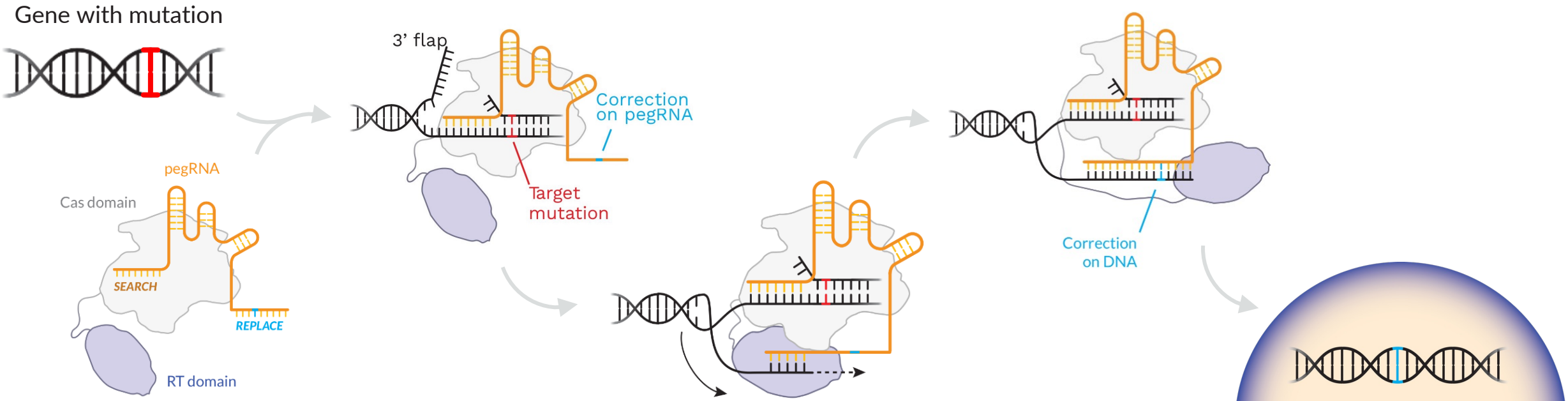

SEARCH

Prime editor complex initiates search for target DNA

FIND & NICK

Prime editor complex finds DNA with target mutation, nicks one strand

PRIME

Nicked DNA strand primes the RT domain for DNA synthesis

REPLACE

Prime editor complex copies in corrective DNA sequence

GENE CORRECTED

3' flap preferentially incorporated[1], excess flap repaired, gene fully corrected

[1] Completion of an edit requires 3 'edit checks,' or places where there has to be a match between the editor and the target DNA

pegRNA = prime editing guide RNA; RT = reverse transcriptase; Cas = CRISPR associated protein

Anzalone, et al (David R. Liu). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 2019.

Prime Editing has the potential to address a wide range of pathogenic variants for therapeutic applications

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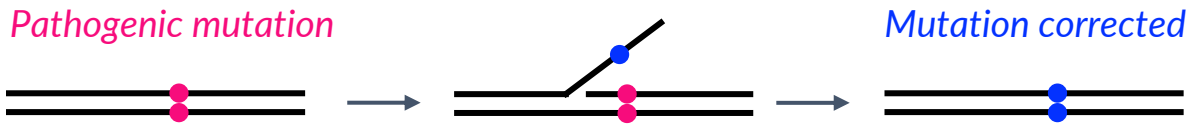
"Single-flap" PE: All 12 possible point mutations, insertions ≥40 bp, deletions ≥80 bp, and combinations thereof[1]

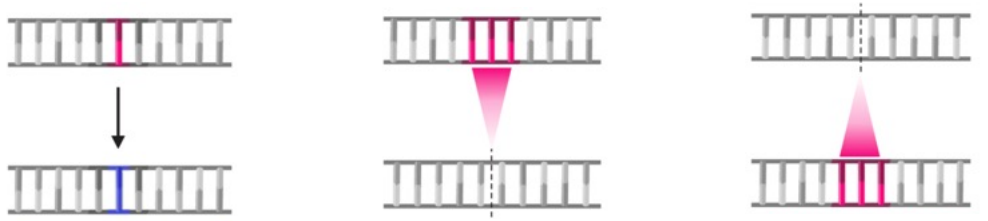


Potential ability to repair ~90% of mutations known to cause genetically driven diseases

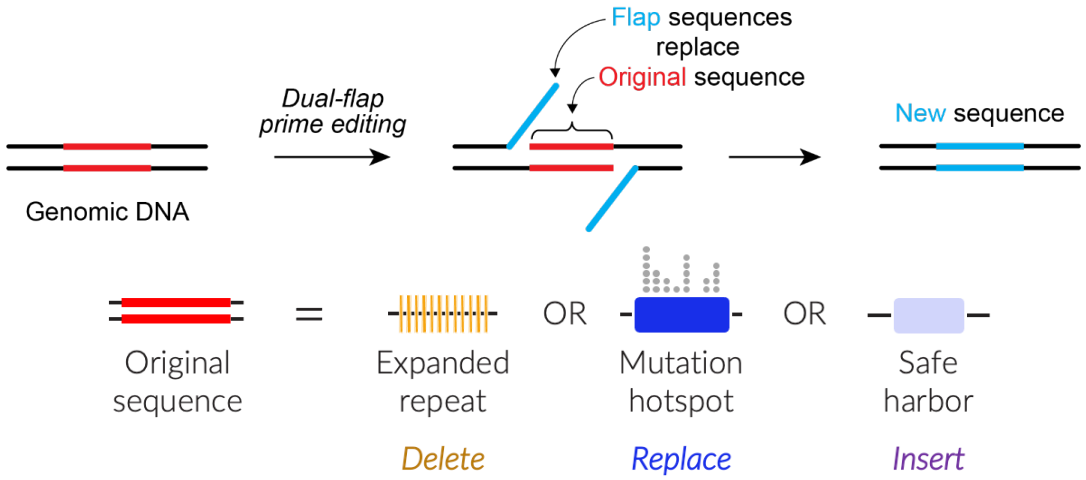
Point Mutation: Mutated base pairs can be substituted

Insertion Mutation: Extra base pairs can be removed

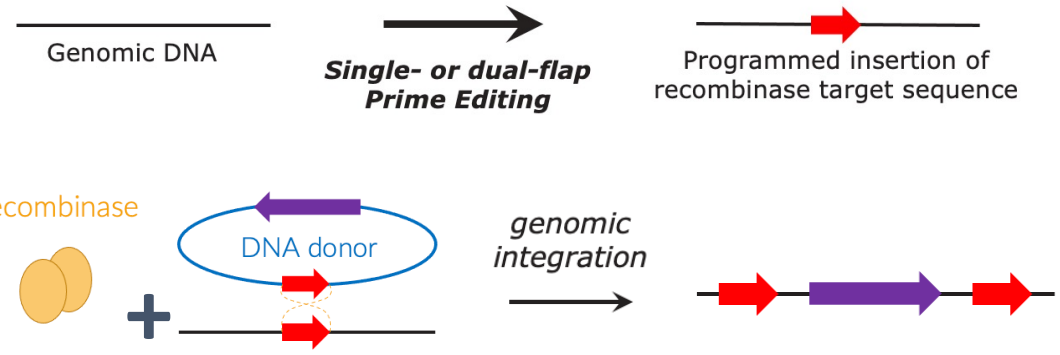
Deletion Mutation: Missing base pairs can be inserted



"Dual-flap" PE: large deletions, replacements, and insertions[2]



PASSIGE: Programmable integration of large genetic payloads[2]



PASSIGE = Prime-Assisted Site-Specific Integrase Gene Editing

[1]Anzalone, et al, *Nature* 2019. [2]Anzalone, et al, *Nat. Biotechnol*. 2021.

Current Prime Medicine portfolio aims to leverage the versatility and breadth of Prime Editing

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To be discussed in detail today

STRATEGIC CATEGORY	TARGET TISSUE	INDICATION	DELIVERY	DISCOVERY	LEAD OPTIMIZATION	IND-ENABLING	Phase 1/2
IMMEDIATE	BLOOD	Chronic Granulomatous Disease	*ex vivo*				
		Fanconi Anemia	*ex vivo*				
	LIVER	Wilson's Disease	LNP				
		Glycogen Storage Disease 1b	LNP				
	EYE	Retinitis Pigmentosa/Rhodopsin	AAV				
		Retinitis Pigmentosa/Usher Syndrome	AAV				
	EAR	Usher Syndrome Type 3	AAV				
		Non-Syndromic Hearing Loss – GJB2	AAV				
DIFFERENTIATION: REPEAT EXPANSION DISEASES	NEURO-MUSCULAR	Friedreich's Ataxia	AAV				
		Myotonic Dystrophy Type 1	viral/non-viral				
		Amyotrophic Lateral Sclerosis	viral/non-viral				
		Oculopharyngeal Muscular Dystrophy	LNP				
		Fragile X Syndrome	viral/non-viral				
		Huntington's Disease	TBD				
	EYE	Fuchs' Endothelial Corneal Dystrophy	viral/non-viral				
DIFFERENTIATION: OTHER	MUSCLE	Duchenne Muscular Dystrophy	AAV				
	LUNG	Cystic Fibrosis	LNP				

PARTNERED PROGRAMS	BLOOD	Sickle Cell Disease (Beam Therapeutics)	*ex vivo*

Targeting repeat expansion diseases with Prime Editing technology

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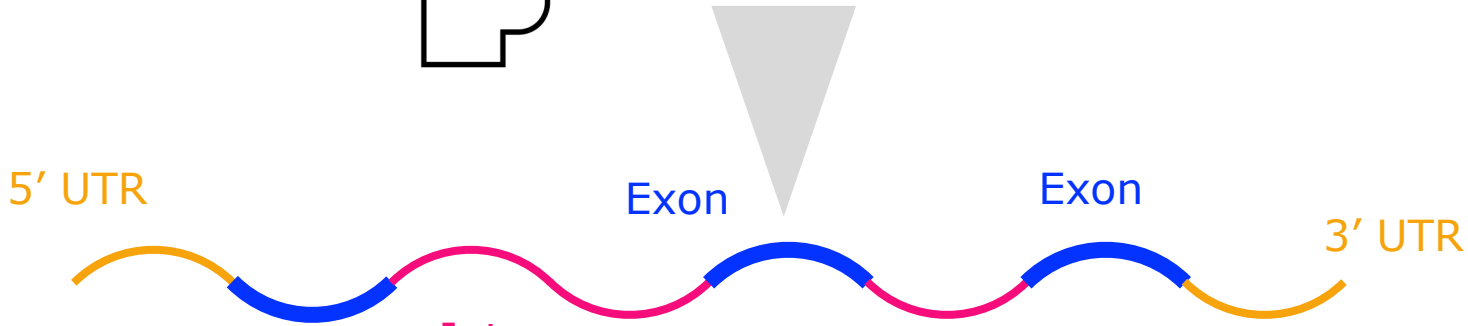
Diverse repeat sequences and variable numbers of repeats can cause disease in multiple tissue types

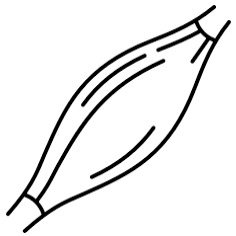
Healthy repeat length

Pathogenic repeat length
(100s to 1,000s of repeats)

Expansion within **EXON**



Expansion within **INTRON**



Expansion within **UTR**

= **CAG** or **CGG** or **CTG** or **GAA** or **GGGGCC** …

Dual-flap Prime Editing approaches to remove pathogenic expanded repeats at endogenous loci

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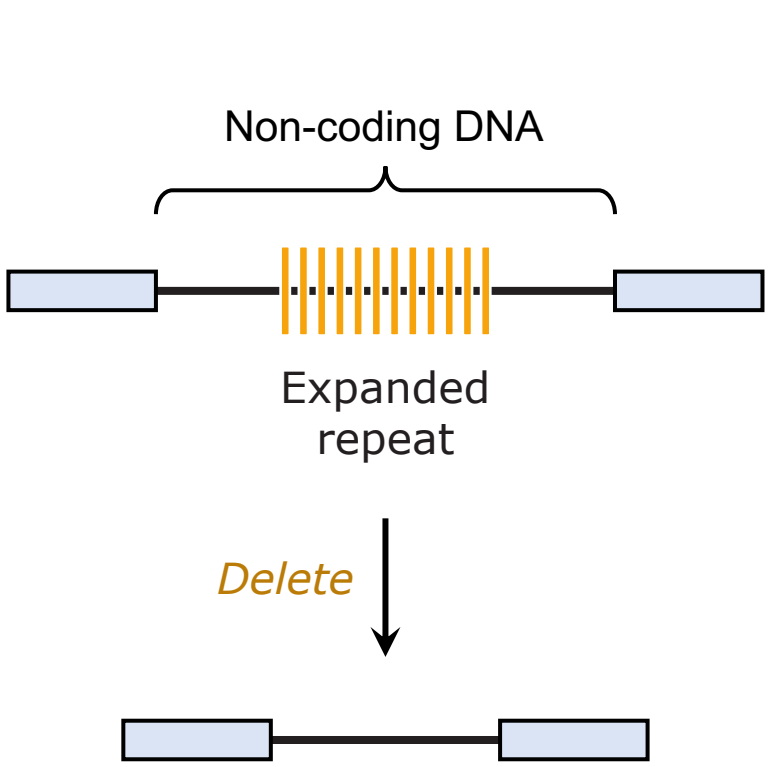
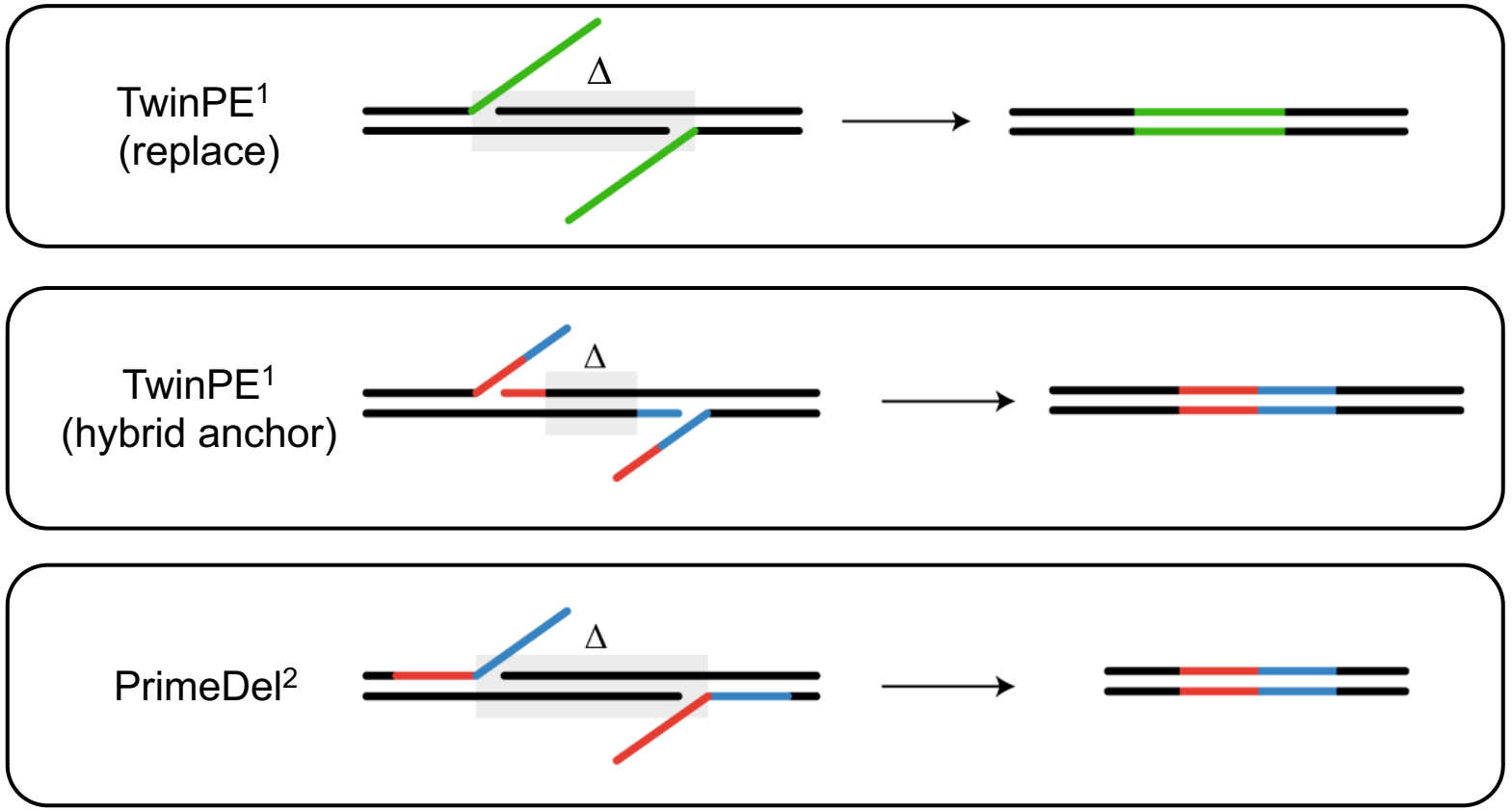
In non-coding regions, multiple dual-flap strategies for removing repeats can be evaluated





Δ = deleted region

and and and = homologous sequences

1. Anzalone, Gao, Podracky, Liu, et al. *Nat. Biotechnol*. 2021
2. Choi, Shendure, et al. *Nat. Biotechnol*. 2021

Friedreich's Ataxia (FRDA) is an autosomal recessive repeat expansion neuromuscular disease

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Clinical manifestations

- Multisystem disorder affecting the nervous system, heart, pancreas, retina
- Gait and limb ataxia due to sensory neuropathy, cerebellar pathology
- Age of onset 5 – 16 years. Mean age of death: 39 years

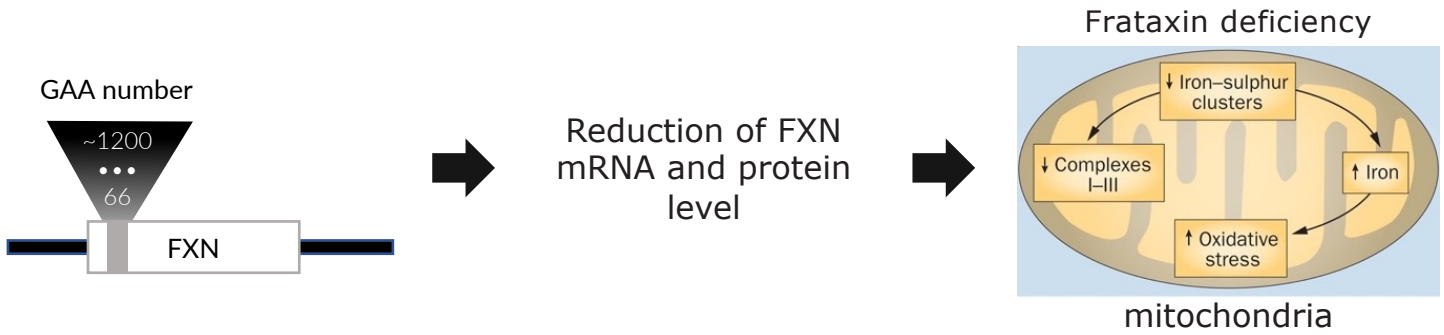
Prevalence
Approximately 4,000 patients in the US, ~15-22k globally

Human biology

- Autosomal recessive: GAA repeat expansion in intron 1 of Frataxin (*FXN*) gene
- Full-penetrance allele carries 66 to 1,200 GAA repeats
- GAA repeat expansion causes decreased *FXN* mRNA and frataxin protein
- Defective iron metabolism causes reduced activity of ISC-containing enzymes in mitochondria, impairing energy generation in sensory neurons and cardiac cells

Prime Medicine's therapeutic approach

- AAV-delivery of Prime Editor to remove pathological GAA repeats from *FXN* and restore frataxin protein expression.



Source: bicycling.com, Bryan Kirkwood

FXN = Frataxin; FRDA = Friedreich's ataxia; ISC = iron-sulfur cluster

Efficient removal of pathogenic repeats in Friedreich's ataxia patient cells by Prime Editors

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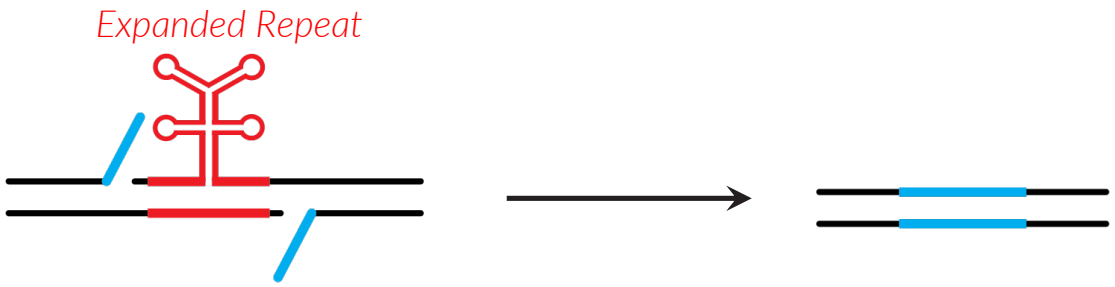
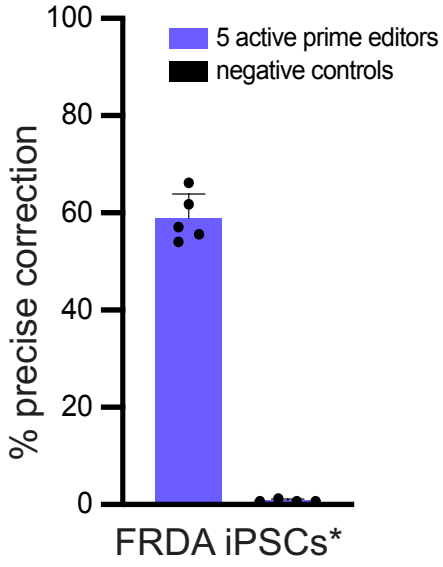
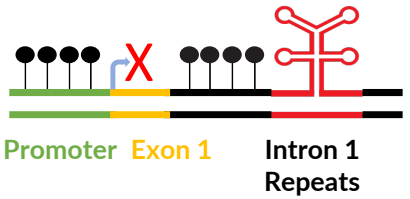
Removal of pathological GAA repeats and hypermethylation at the Frataxin (*FXN*) gene in Friedreich's Ataxia patient cells

Dual-flap Prime Editing loops out expanded repeat



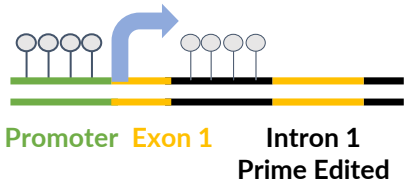


Hypermethylation prevents mRNA transcription of *FXN*

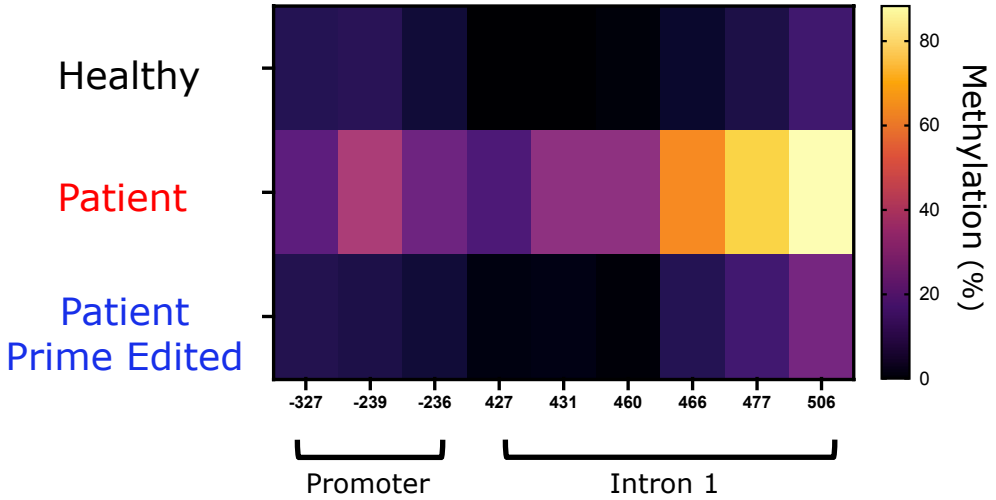


Prime Editing

Demethylation by Prime Editing restores mRNA transcription of *FXN*



Removal of Hypermethylation[1]



Many repeat expansion diseases exhibit hypermethylation as a key feature of the underlying pathogenesis

Conclusions

- ✓ High efficiency, very precise editing of patient cells without double strand breaks
- ✓ Restores normal methylation of FXN gene

FXN: frataxin gene: FRDA: Friedreich's ataxia; iPSCs: induced pluripotent stem cells. 1. Methylation quantified by bisulfite sequencing

Dual-flap Prime Editors completely restore total *FXN* mRNA expression and isoforms in FRDA patient cells

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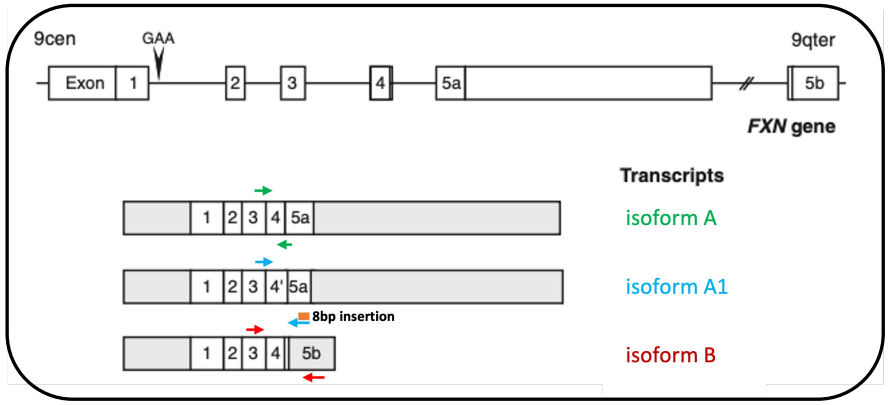
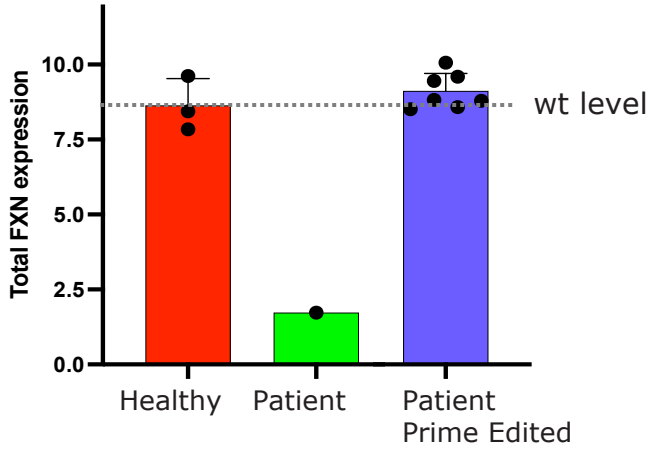
Expression of transcript isoforms is restored after removal of repeats from *FXN* intron 1



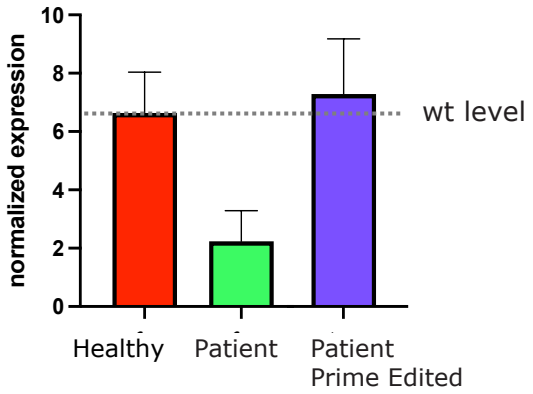
Restoration of total *FXN* mRNA expression in iPSCs

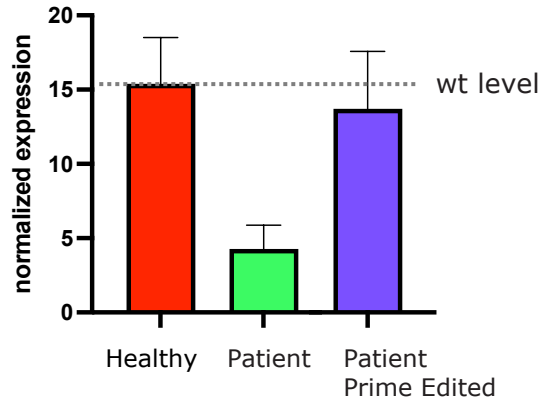


Cardiomyocytes

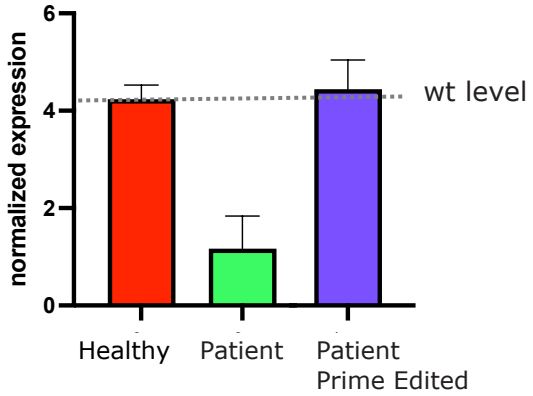
iDRGs

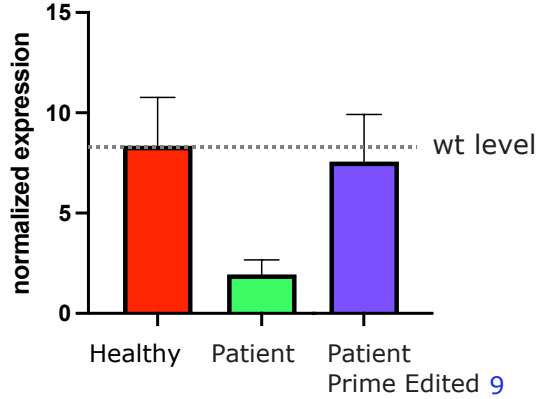
Total *FXN* mRNA





FXN isoform A





FXN: Frataxin; FRDA: Friedreich's Ataxia; iDRG: iPSC-derived dorsal root ganglia

Prime Editors efficiently remove pathogenic repeats in Friedreich's ataxia (FRDA) patient cells and restore frataxin function

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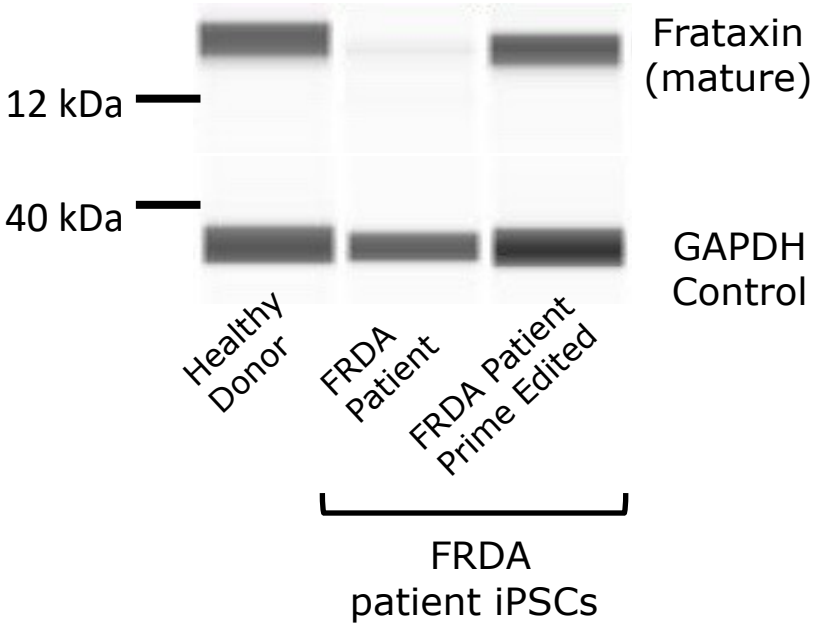
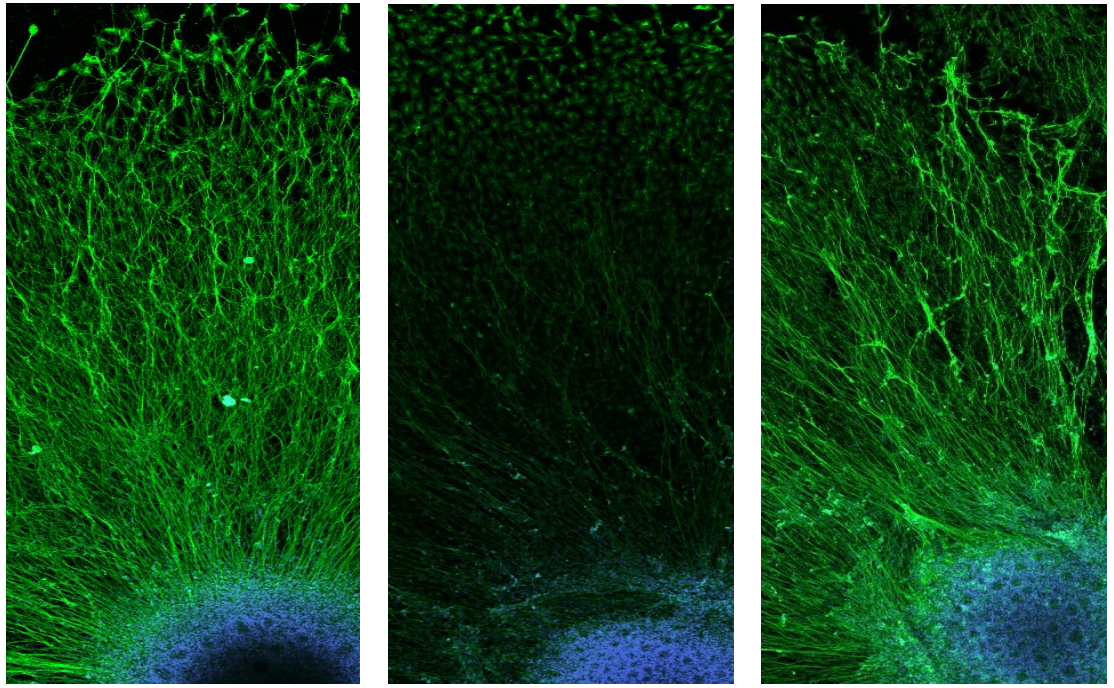
High-efficiency Prime Editing restores FXN protein expression and sensory neuron phenotype in Friedreich's Ataxia patients' dorsal root ganglia organoids

Restoration of Frataxin protein expression after delivery of Prime Editor



Restoration of axonal projections after Prime Editor correction

βIII-TUB
DAPI



Healthy Donor | FRDA Patient | FRDA Patient Prime-Edited

FXN: Frataxin; FRDA: Friedreich's Ataxia; iDRG: iPSC-derived dorsal root ganglia. DAPI: nuclear staining; βIII-TUB: axonal projection staining

Prime Editors are efficient when delivered by dual-AAV to the CNS

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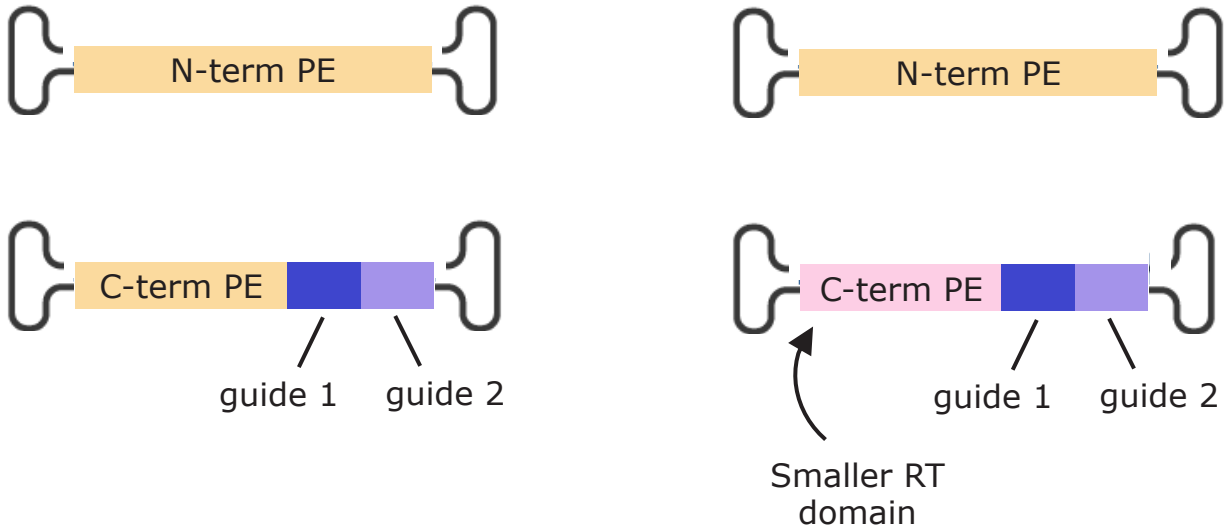
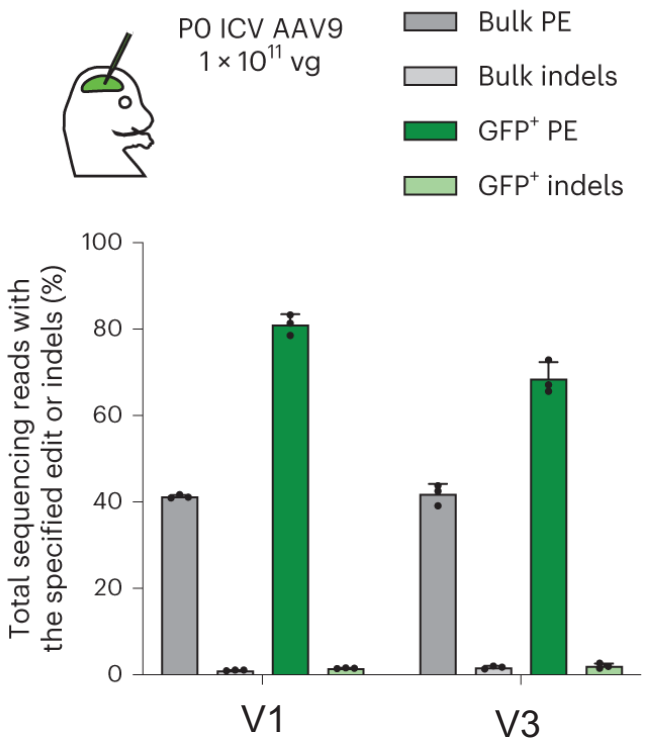
Engineering the Prime Editor AAV genome to deliver the Prime Editor efficiently in mice

AAV genome architectures





Davis, Banskota, Liu, et al. *Nat. Biotechnol.* (2023).

Prime Editing Delivery: CSF and local administration to CNS via dual-AAV achieves high efficiency in mouse brain

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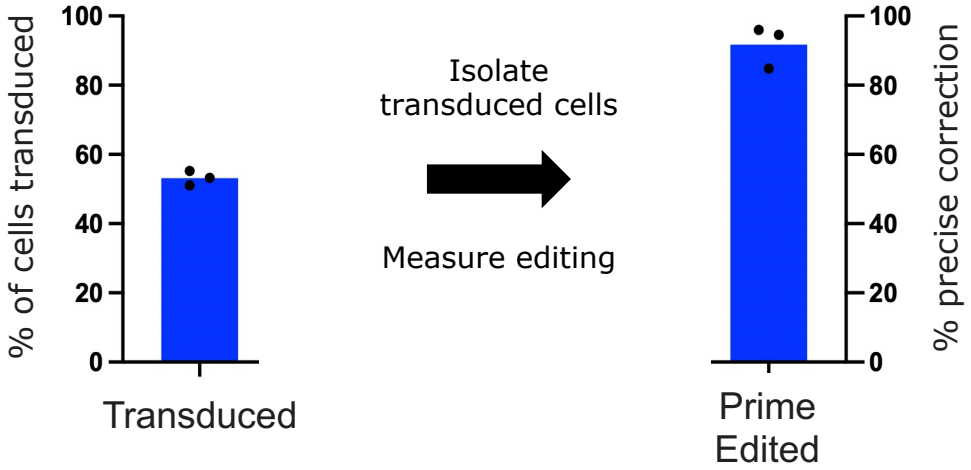
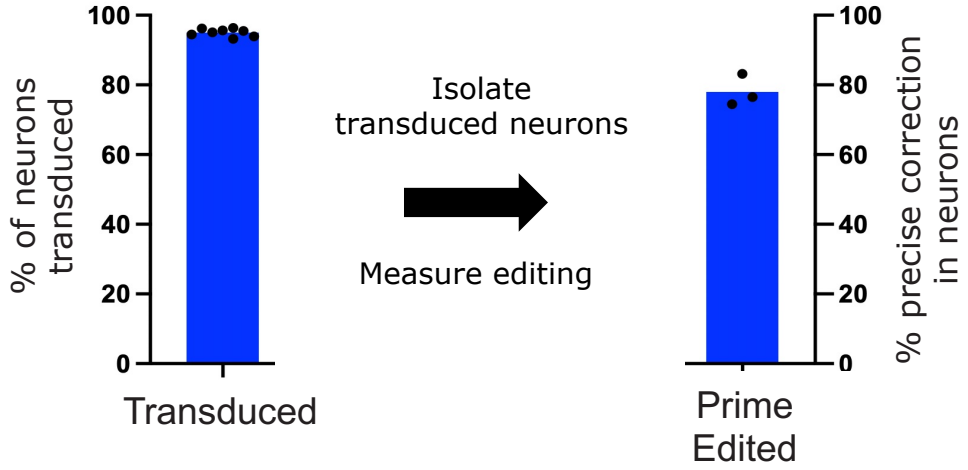
Dual-AAV[2] effectively delivers to ~ 95%, and precisely edits ~80%, of neurons in adult mice

Prime Editor dual-AAV
- Prime Editor
- Prime editor dual guide RNA

Neonatal mice – ICV infusion[1]
transduced cortex (left) and precisely edited cortical cells (right)



Adult mice – local administration[1]
transduced neurons (left) and precisely edited neurons[2] (right)



Further optimizations to the AAV genome including neuron-specific promoters conducted at Prime Medicine

[1]Three weeks in neonatal mice via intra-cerebral infusion (ICV); 5 weeks in adult mice via local administration into cerebellum or cortex. [2]Prime Editor cassette with neuron-specific promoter. All experiments shown are Proof of Concept delivery experiments using a control Prime Editor site. Davis, Banskota, Liu, et al. *Nat. Biotechnol.* 2023.

Engineered Prime Editor proteins markedly improve editing efficiency *in vivo* when delivered by dual-AAV

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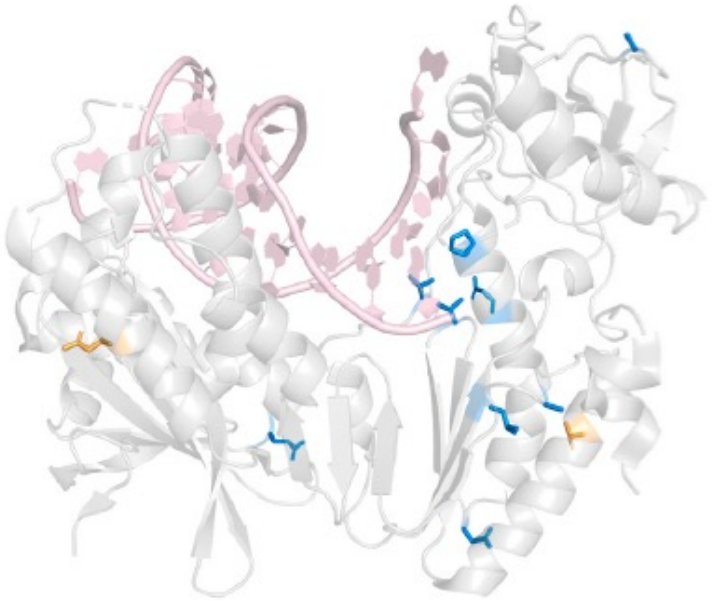
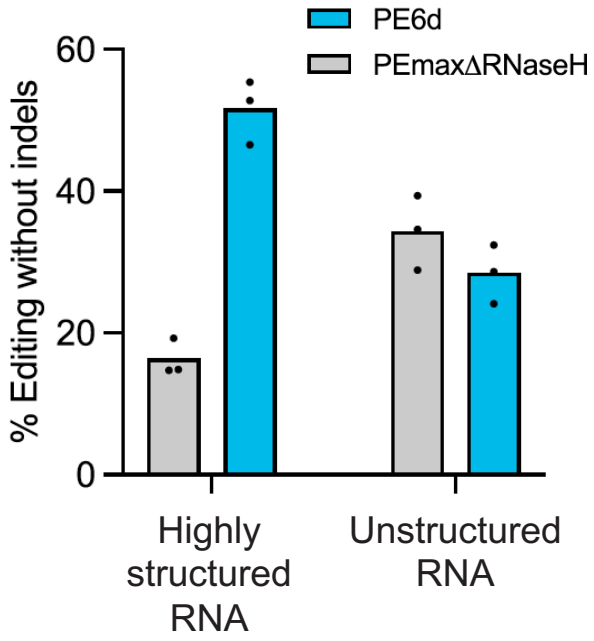
PE6 proteins package better in AAV and improve long-flap and dual-flap editing efficiency

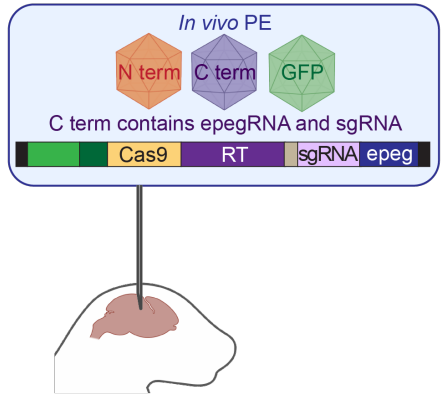
Prime Editor protein engineered using directed evolution

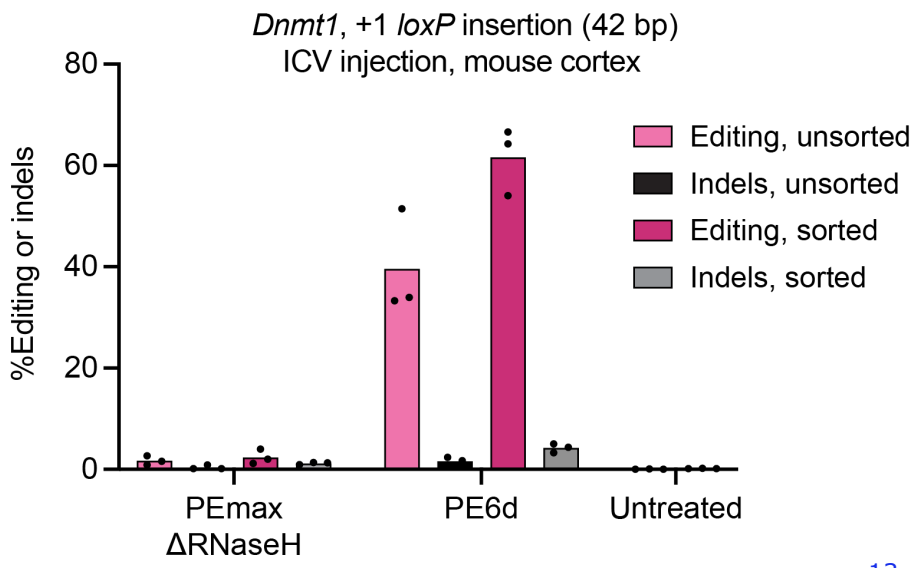


MMLV RT

PE6d protein improves Prime Editing with long, structured RNA templates and leads to marked improvement in *in vivo* editing efficiency







Doman, Pandey, Liu, et al. *Cell* **186**, 3983-4002 (2023).

Studies ongoing to evaluate dual-AAV Prime Editors in fully humanized mouse model of FRDA and in large animals

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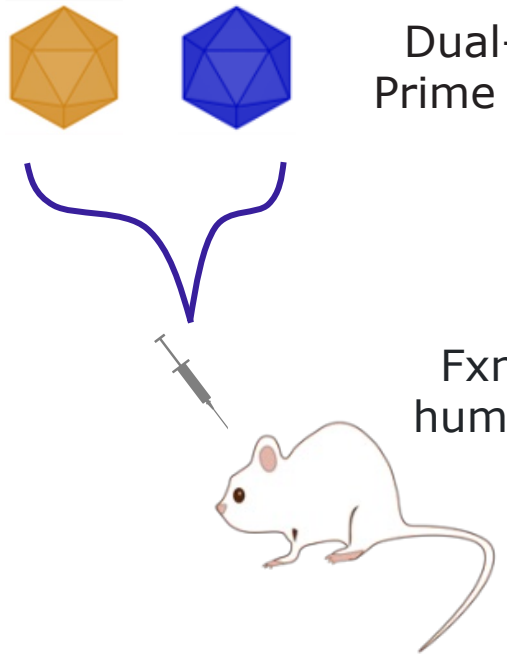
Potential routes of administration for patients are under evaluation

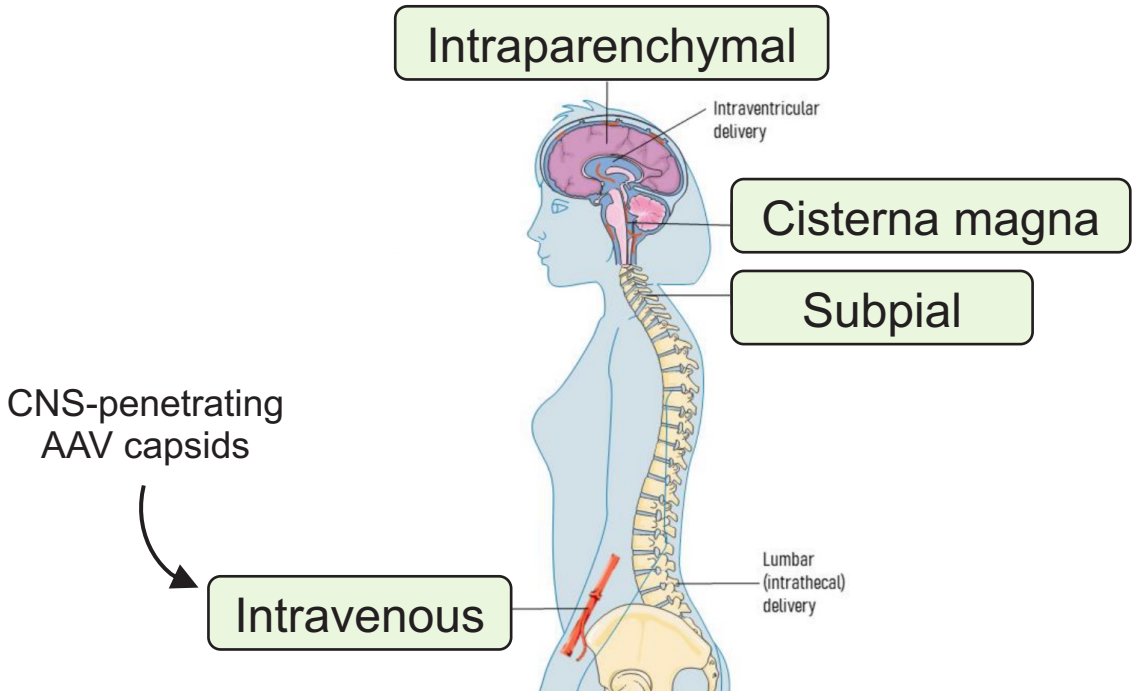
Evaluation of Prime Editors in FRDA mouse model containing >800 GAA repeats



Dual-AAV
Prime Editor

Fxn^{null}::YG8s$(GAA)_{>800}$
humanized mouse model

Evaluating multiple routes of administration for delivery to the CNS



Fxn^{null}::YG8s$(GAA)_{>800}$ = humanized mouse model of FRDA null for mouse *Fxn* and carrying human *FXN* transgene with expanded GAA repeat; FRDA = Friedreich's ataxia

Fragile X Syndrome (FXS) is an X-linked dominant neurodevelopmental disorder

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Clinical manifestations

- Developmental delay, intellectual disability, anxiety, autism spectrum disorder
- No structural or progressive damage to CNS
- Diagnosis is typically at 3-4 years of age; life expectancy not affected

Human biology

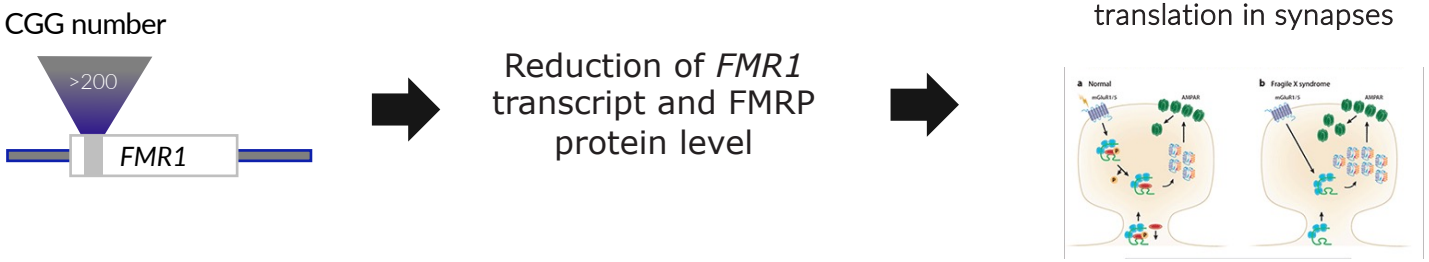
- X-linked dominant: CGG expansion (>200 repeats) in the 5' UTR of *FMR1* gene leads to aberrant hypermethylation of the promoter and loss of transcription
- *FMR1* encodes FMRP, a protein essential for normal synaptic plasticity & function
- Disease severity is dependent on *FMR1* CGG repeat length and methylation status

Prime Medicine's therapeutic approach

- AAV-delivery of Prime Editor to remove pathological CGG repeats from *FMR1* and restore FMRP protein expression. Target brain regions include caudate nucleus, hippocampus and various areas of the cortex.

Prevalence
Approximately 40,000 to 60,000 patients in the United States

From Hagerman, et al. PMID: 28960184



FMR1 = Fragile X Messenger Ribonucleoprotein 1; FMRP = Fragile X Messenger Ribonucleoprotein; CNS = Central Nervous System; AAV = Adeno-Associated Virus

Expansion of CGG repeats in *FMR1* leads to methylation and loss of FMRP protein expression

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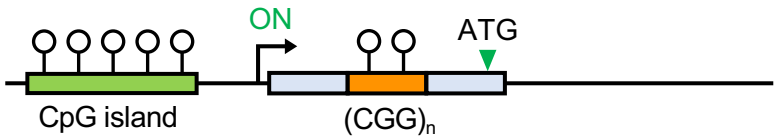
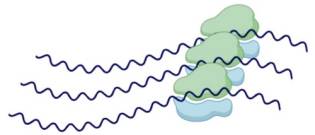
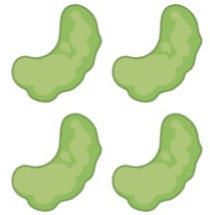
FMR1 $(CGG)_n$ Genotype	*FMR1* mRNA	FMRP protein

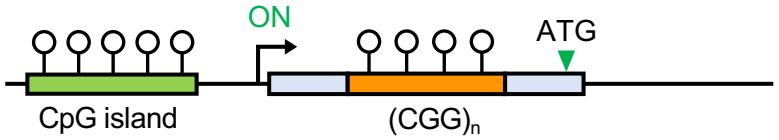
Healthy (n < 55)

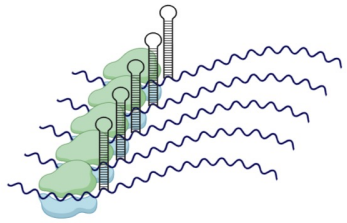


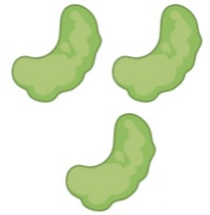




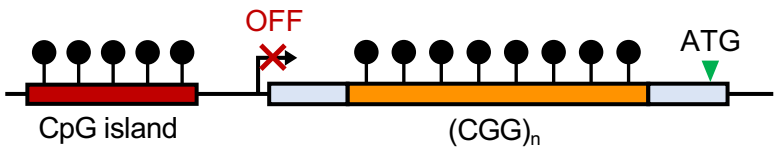
Pre-mutation, risk of FXPOI/FXTAS (55 ≤ n ≤ 200)

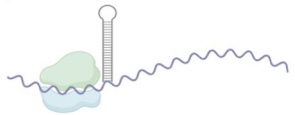






Fragile X Syndrome (Full mutation; n > 200)





FXTAS: Fragile X-associated tremor/ataxia syndrome; FXPOI: Fragile X-associated primary ovarian insufficiency; *FMR1*/FMRP = Fragile X Messenger Ribonucleoprotein mRNA/protein

Full reactivation of *FMR1* transcription and translation in patient cells following delivery of a Prime Editor*

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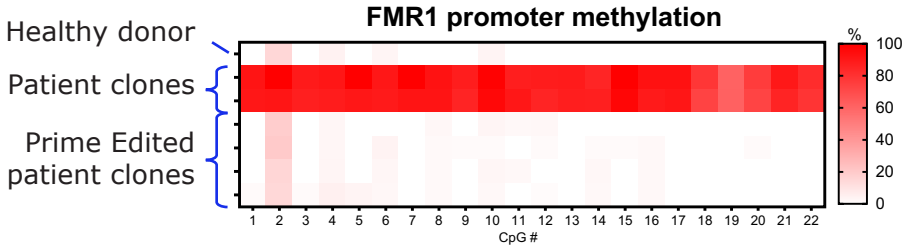
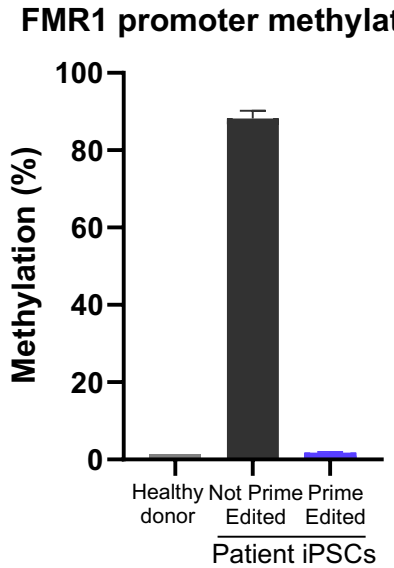
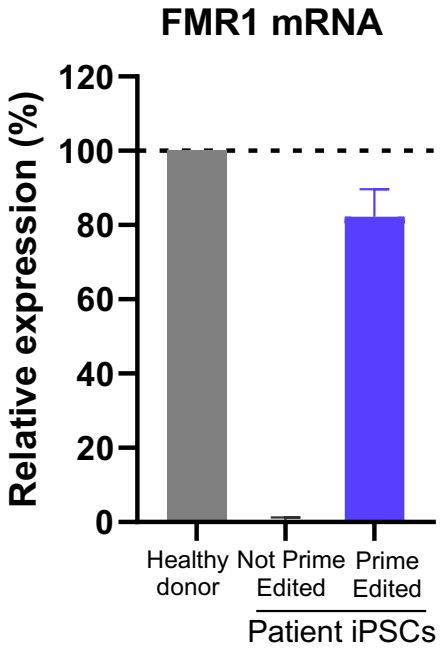
Prime Editor removes CGG repeats, restores normal methylation, *FRM1* mRNA, and FMRP protein levels

Loss of methylation of *FMR1* promoter after Prime Editor correction

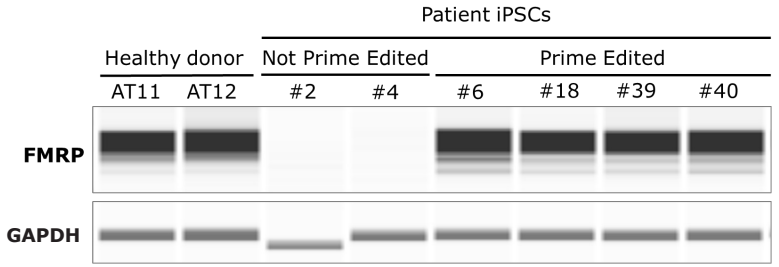


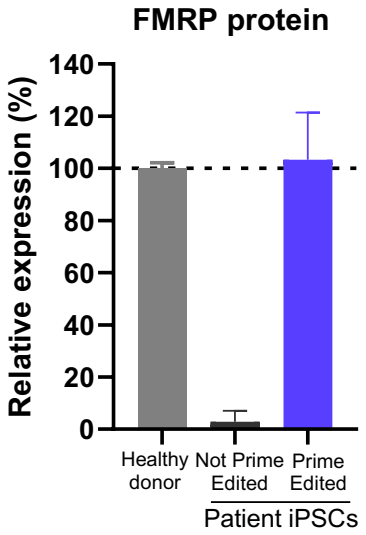


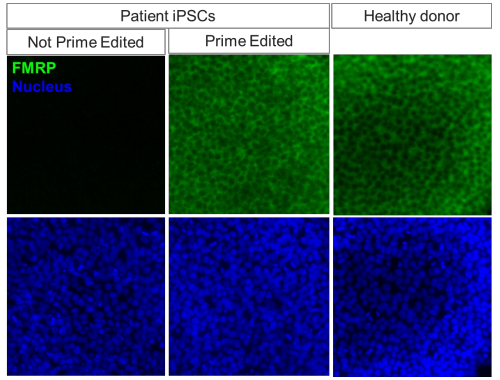
FMR1 mRNA restored after Prime Editor



FMRP protein expression restored after Prime Editor correction







**Edited and unedited patient clones following delivery of a Prime Editor. FMR1* = Fragile X Messenger Ribonucleoprotein 1; FMRP = Fragile X Messenger Ribonucleoprotein

Leveraging a modular dual-AAV platform for delivery of Prime Editors to the CNS

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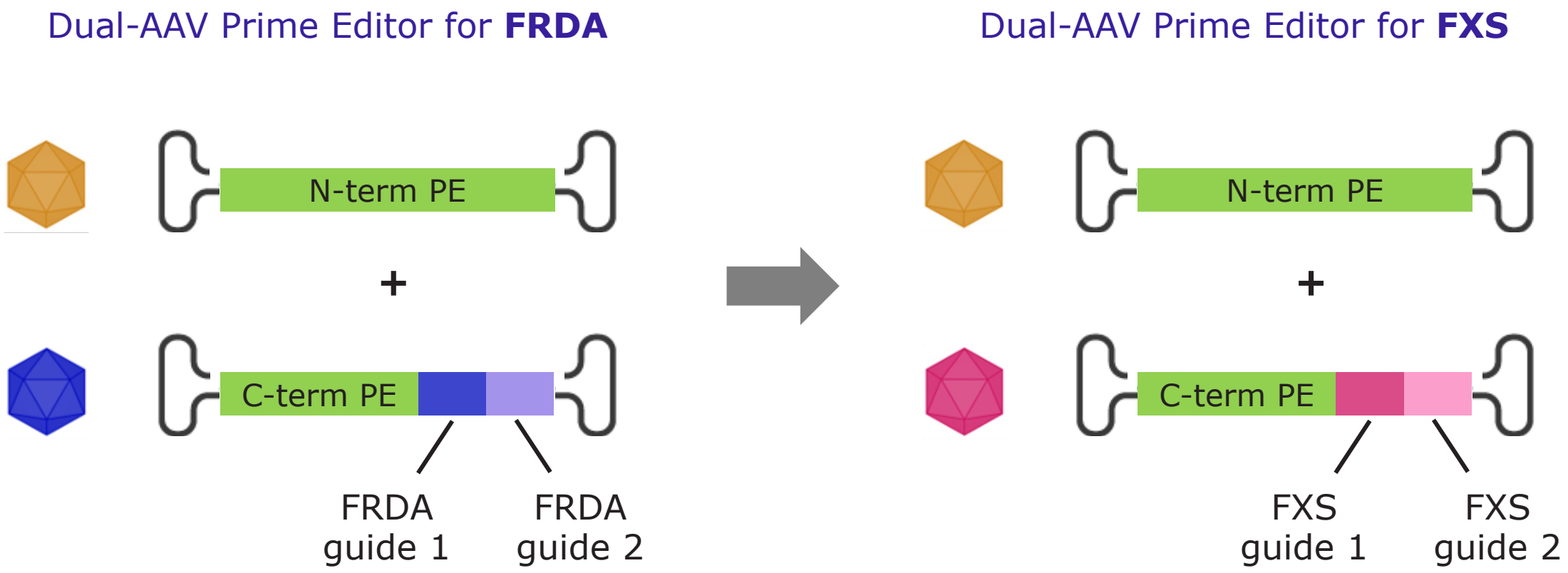
Potential to create new Prime Editor dual-AAVs by swapping guide RNAs in otherwise constant AAV genomes (promoters, coding sequences, regulatory elements) and capsids



FRDA = Friedreich's ataxia; FXS = Fragile X syndrome

Myotonic dystrophy type 1 (DM1) is an autosomal dominant repeat expansion disease

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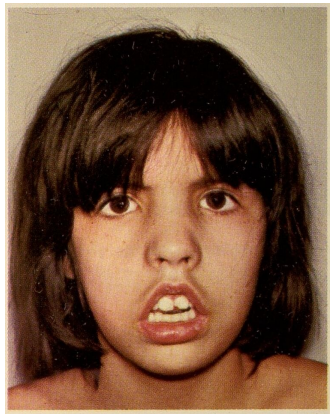
Clinical manifestations

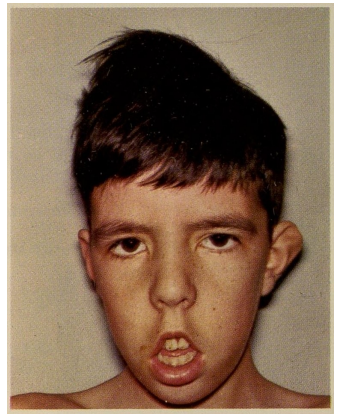
- Multisystem disorder with progressive distal and facial muscle weakness, myotonia, cardiac conduction abnormalities, cataracts
- Mild DM1 (mild myotonia, normal life span); Classic DM1 (adults may become disabled and may have shortened life span); Congenital DM1 (severe at birth, also intellectual disability common, early death)

Prevalence
Approximately 1 in 2,300 people in the US (all subtypes)

Human biology

- Autosomal dominant: CTG expansion (>50 repeats) in the 3' UTR of *DMPK* gene
- Altered expression of DMPK and neighboring genes, sequestration of splicing components in RNA foci results in spliceopathy
- CTG repeat length correlates inversely with age of onset, parallels severity

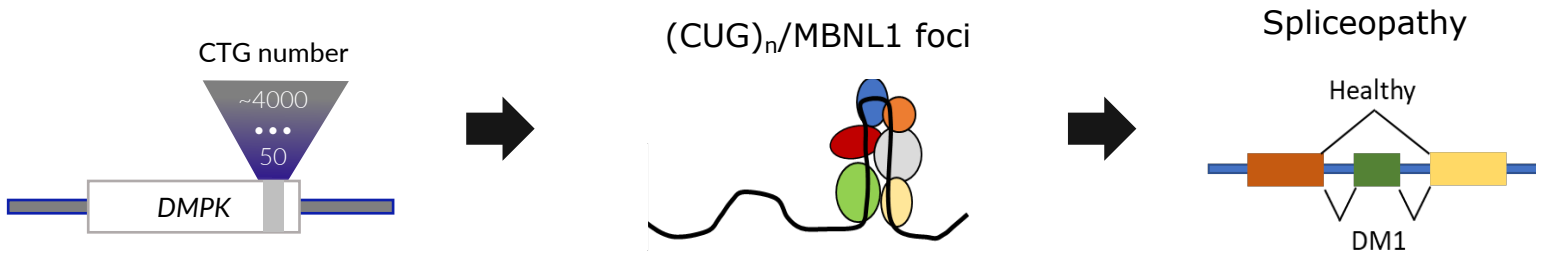




Source: wikipedia.com

Prime Medicine's therapeutic approach

- AAV-delivery of Prime Editor to skeletal and cardiac muscle to remove pathological CTG repeats from *DMPK* and to eliminate RNA foci and restore normal gene expression



DMPK = dystrophia myotonica protein kinase; MBNL1 = muscleblind like splicing regulator 1; AAV = Adeno-Associated Virus

Removal of pathological expanded repeat in 3’ UTR of *DMPK* with Prime Editor reduces toxic RNA-foci in patient cells

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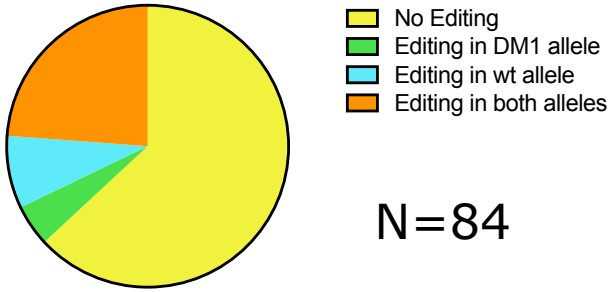
Prime Editing can remove expanded CTG repeats, correct RNA foci, and does not affect expression of *DMPK* or *SIX5*

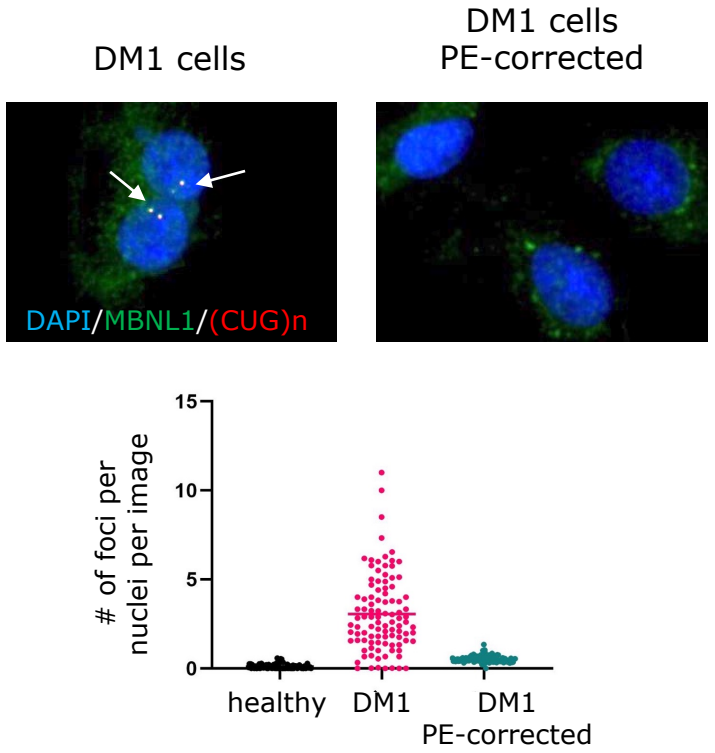
Clonal analysis demonstrates editing of expanded CTG alleles



	# of clonal lines
No Editing	53
Editing of DM1 allele only	4
Editing of wt allele only	7
Editing of both alleles	20
Effective % Editing	28.6%

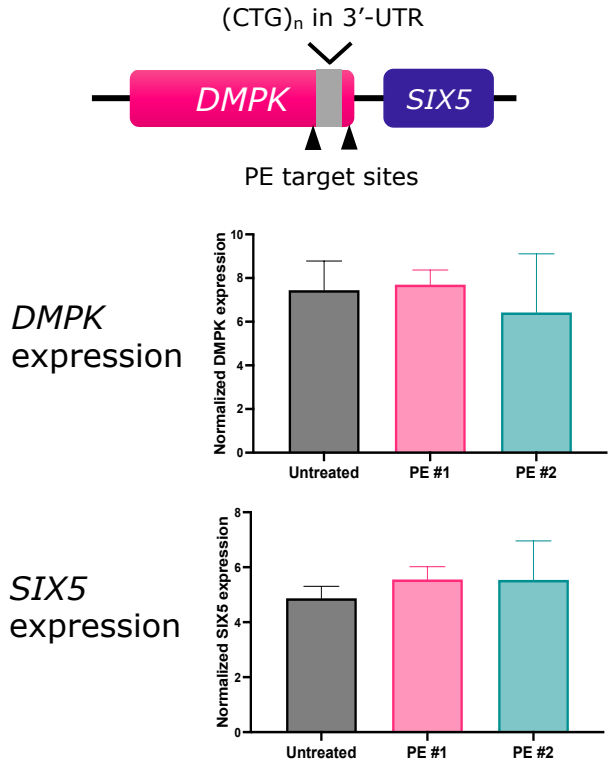
Patient-derived iPSCs

Prime Editor leads to significant reduction of toxic RNA foci



PE-corrected cardiomyocytes differentiated from iPSCs

PE does not affect expression of *DMPK* or neighboring *SIX5*



Prime-edited HEK293T cells

DMPK = myotonic dystrophy protein kinase; MBNL1 = muscleblind like splicing regulator 1; *SIX5* = six homeobox 5; PE = Prime Editor

Dose-responsive precise editing following dual-AAV delivery to mouse skeletal myotubes

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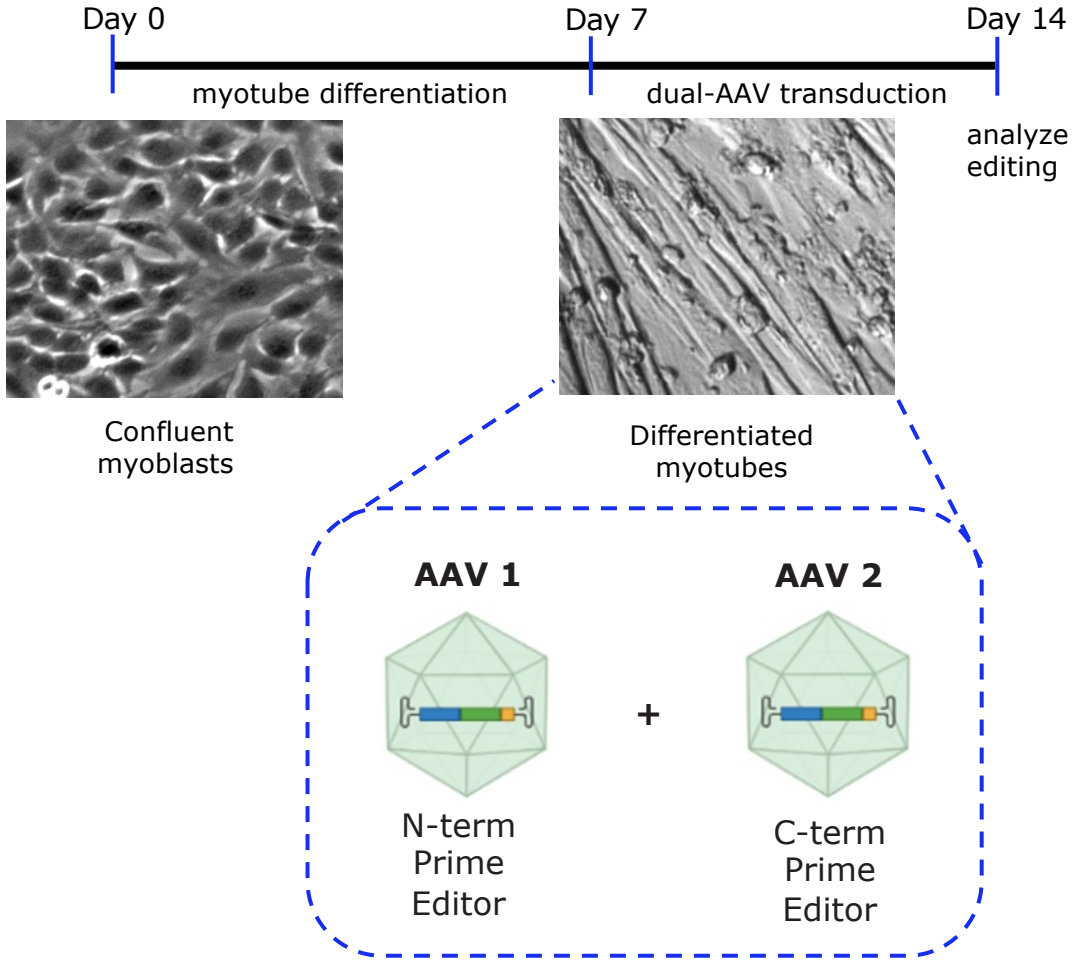
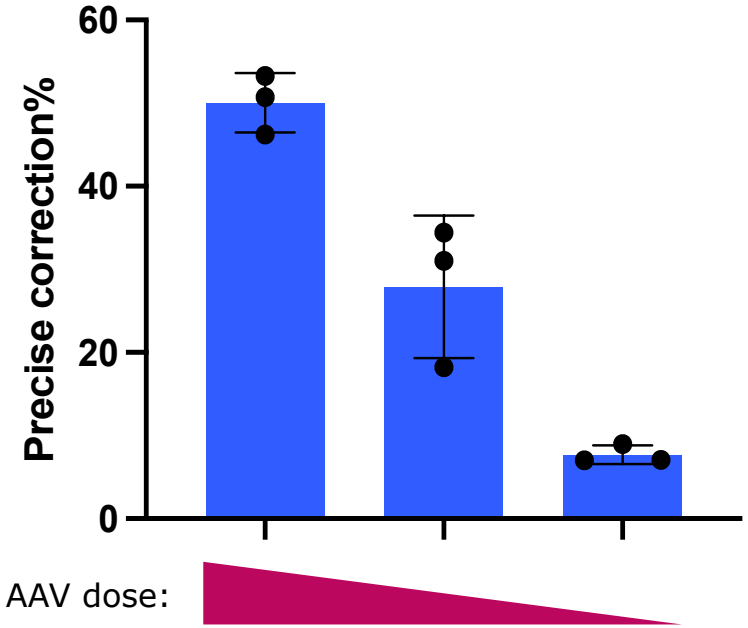
Dose-dependent editing in mouse myotubes via AAV6-mediated Prime Editor delivery



Dose-dependent Prime Editor correction in mouse skeletal myotubes



Prime Editing at test locus

AAVs can safely deliver gene therapies to skeletal muscle of patients

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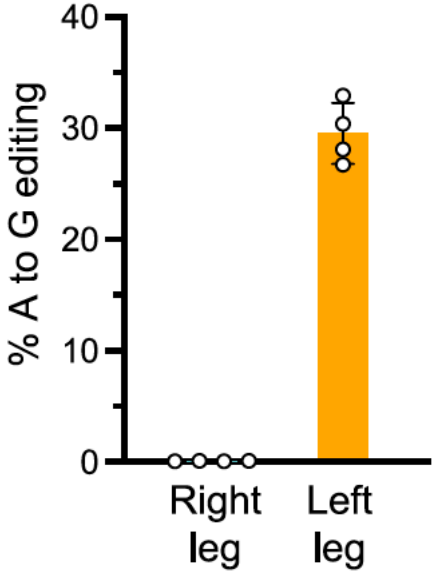
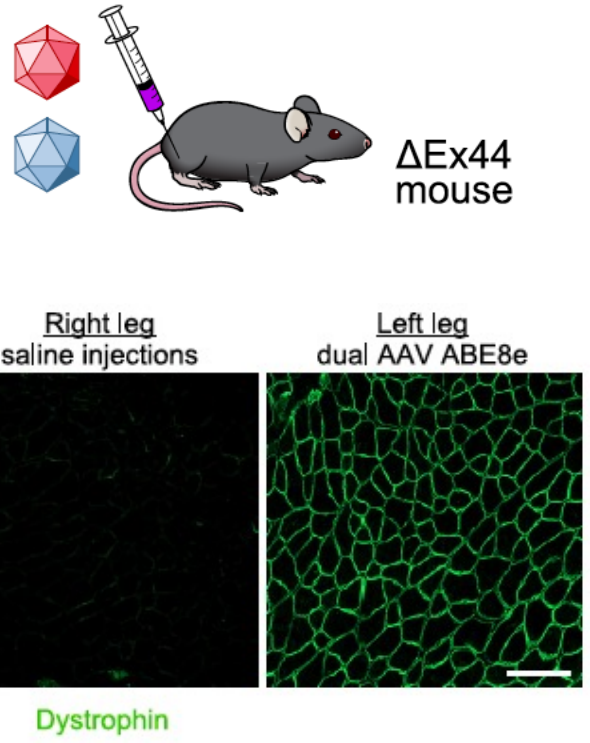
Both skeletal and cardiac muscle can be transduced by AAV

FDA approval of micro-dystrophin AAV gene therapy for Duchenne muscular dystrophy

Proof of concept dual-AAV editing in skeletal muscle in DMD mouse





Chai, Olson, et al. *Mol Ther Nucl Acids* (2023); DMD = Duchenne muscular dystrophy.

Summary

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- Prime Editing is a programmable, specific, precise, versatile and efficient gene editing approach that has the potential to address a large percentage of genetic variants associated with disease.
- Dual-flap Prime Editing approaches offer editing flexibility and can be used to excise pathogenic expanded repeats at their endogenous genetic loci.
- Prime Editors can remove pathogenic CGG repeats from *FMR1* to restore healthy methylation patterns and FMRP expression in cells derived from Fragile X syndrome patients.
- Prime Editors can remove pathogenic GAA repeats from *FXN* to restore healthy methylation patterns and gene expression in cells derived from Friedreich's ataxia patients, restoring axonal projections in dorsal root ganglion organoids generated from patient cells.
- Prime Editors can remove pathogenic CTG repeats from *DMPK* in cells derived from patients with myotonic dystrophy type I and reduce toxic RNA foci in differentiated cardiomyocytes.
- Using dual-AAV delivery, Prime Editors can achieve high editing efficiency in the CNS of mice *in vivo* and in cultured skeletal muscle myotubes.

THANK YOU!

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Prime Medicine Research & Development