Gene Therapy for Rett Syndrome using Editing Technologies

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BACKGROUND

RETT SYNDROME

Rett syndrome is a rare, X-linked neurodevelopmental disorder caused by mutations in the Methyl-CpG binding Protein 2 (***MECP2***) gene affecting 1 in 10,000 girls. Symptoms present at 6-18 months and include reversion of learnt behaviours, speech and purposeful movement and are life-lasting.

GENE THERAPY

Conventional Gene Therapy

Conventional gene therapy for Rett syndrome is challenged by the regulation of *MECP2* expression spatially and temporally by the highly important 5' and 3' endogenous regulatory elements. Retaining physiological expression of *MECP2* under these control elements is crucial.

Gene Editing Therapy

Clustered regularly interspersed palindromic repeats (CRISPR) is a novel gene editing tool currently being tested in over 20 clinical trials. Cas9 endonuclease initiates a break in the DNA guided by single guide RNA (sgRNA) sequences implementing repair machinery and an opportunity for gene editing.

Exon Replacement Therapy

A novel gene therapy using CRISPR mediated homology independent targeted integration (HITI) to replace mutant exons of *MECP2* with wild type copies at the native locus retaining endogenous expression of *MECP2*.

AIMS & HYPOTHESIS

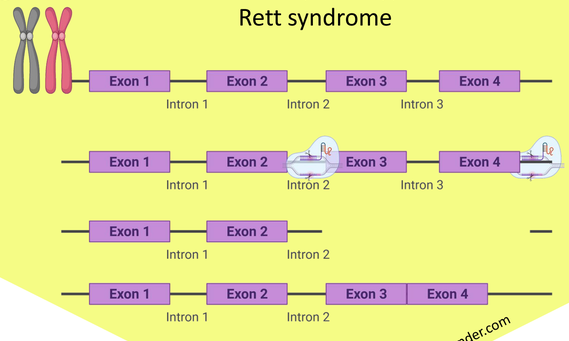
Hypothesis

Novel targeted gene replacement editing therapies can be used to correct pathogenic mutations causing Rett syndrome in *MECP2.*

Aims

1. To determine the efficiency of gene replacement therapy to correct pathogenic mutations in *in vitro* cell cultures of patient derived cells.

2. To assess the efficiency of gene replacement therapy to correct pathogenic mutations and reverse phenotypes in *in vivo* mice models of Rett syndrome



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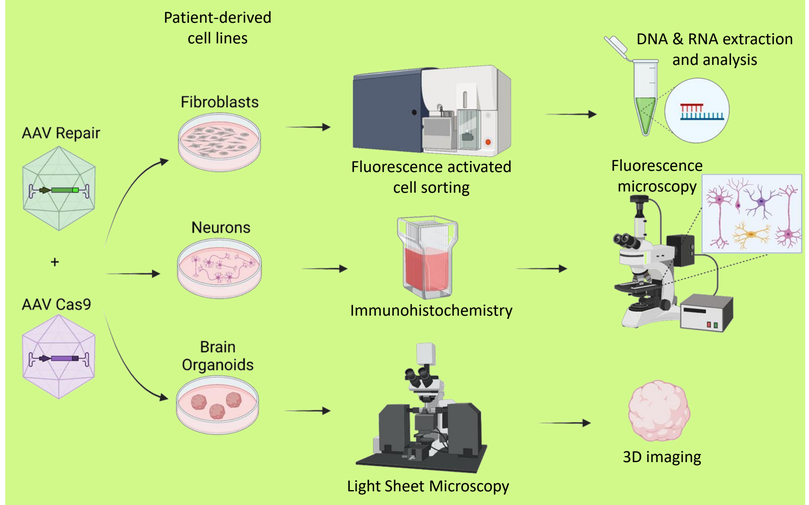
MATERIALS & METHODS

In Vitro Methods



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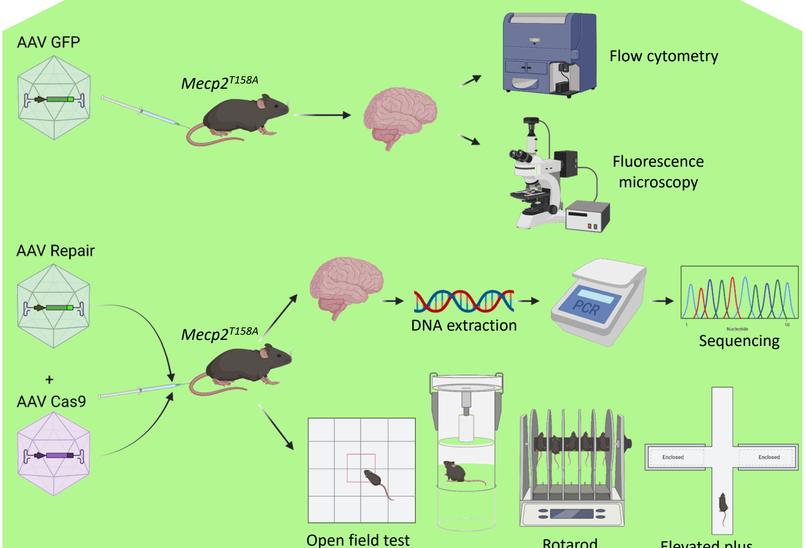
In Vivo Methods



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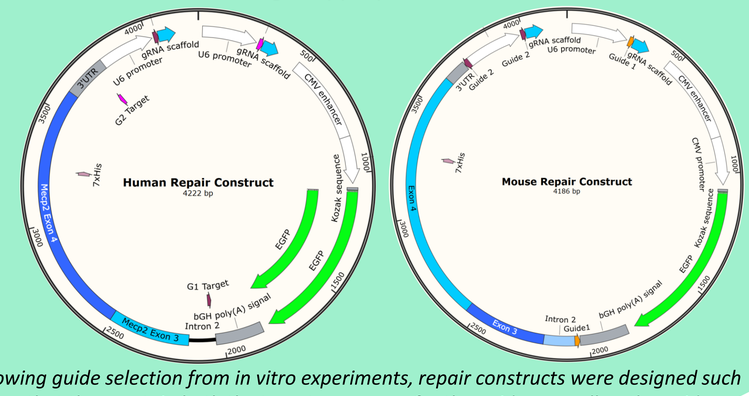
RESULTS

Construct design

Guides upstream of exon 3 and downstream of exon 4 were designed and tested in cell lines prior to selection for the final construct. The final constructs, designed using SnapGene® were ordered and cloned into inverted terminal repeat (ITR) containing plasmids for AAV packaging prior to testing in appropriate models

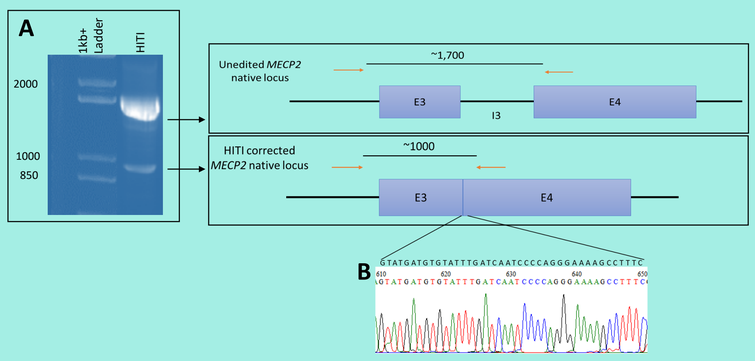


Following guide selection from in vitro experiments, repair constructs were designed such that they contain both the target sequences for the guides as well as the guides and scaffolds. This design allows only for editing if the repair construct is present. The fluorescent tag enhanced GFP is also included to determine transduction efficiency in cells.

RESULTS

Exon Replacement in HEK293 cells

Exons 3 and 4 were excised and replaced at the native locus of the *MECP2* gene using Cas9 and a repair construct to perform HITI.

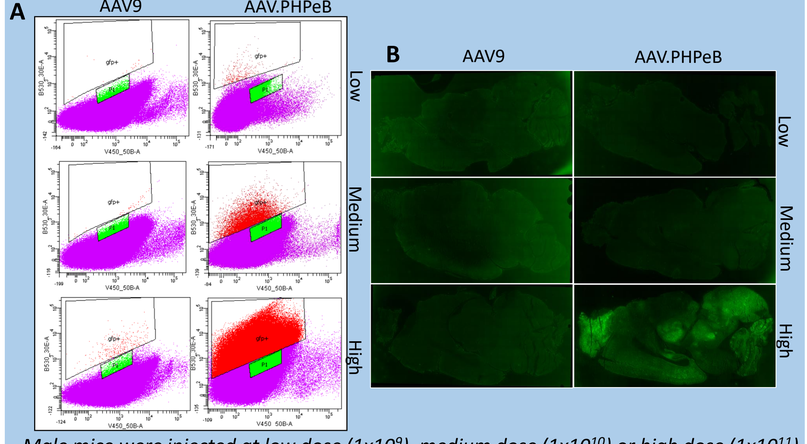


The HITI construct and PX459 containing Cas9 were co-transfected into HEK cells to remove exons 3 and 4 and replacing with a fused wild type copy. After puromycin selection, DNA was harvested, amplified by PCR and size separated by gel electrophoresis (A) and confirmed by sanger sequencing (B).

RESULTS

AAV Dosage in *Mecp2*^{T158A} Mice

Dosage for subsequential experiments were determined by assessing the efficiency of three different doses of two AAV capsids in *Mecp2*^{T158A} mice.



Male mice were injected at low dose (1x10^{9}), medium dose (1x10^{10}) or high dose (1x10^{11}). Two capsids, AAV9 and AAV.PHPeB were tested for downstream experiments. Brains were extracted and FAC sorted based on GFP to determine the most effective dose required (A). Microscopy was also used to assess regional transduction (B).

CONCLUSIONS

- **Gene replacement editing therapy poses a possible solution to overcome the regulatory constraints associated with *MECP2* expression**
- **Human and murine replacement AAV-constructs have been developed and tested**
- **Replacement has been demonstrated in human cell models**
- **Dosage of AAV has been determined in mice models**

FUTURE DIRECTIONS

- **Human constructs will be tested in more appropriate cell models such as patient iPSC-derived neurons and organoids**
- **Mouse constructs will be tested in a Rett syndrome mouse model to determine correction efficiency at genomic level using NGS sequencing**
- **Mouse constructs will be tested in a Rett syndrome mouse model to assess phenotypic rescue using specialised behavioural equipment**
- **Toxicity and side effects of replacement editing therapy will be assessed**

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