Reversion of Rett Syndrome Phenotype Following AAV9/*MECP2* Gene Transfer to Neonatal *MECP2*-Null Mice

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INTRODUCTION

Rett Syndrome (RTT) is an X-linked autism-spectrum disorder affecting 1 in every 10,000 female births. It is characterized by a normal development followed by an early neurological regression that severely affects motor, cognitive and communication skills. Over 95% of typical RTT is caused by mutations in the gene encoding the transcriptional modulator Methyl-CpG-Binding Protein 2 (*MECP2*) located in Xq28[2]. MeCP2 acts by binding to methylated CG dinucleotides in some gene promoters, ultimately causing chromatin compactation leading to gene silencing. Currently, there is not any curative therapy for Rett syndrome, and current management remains symptomatic. Exogenous delivery of MeCP2 following gene therapy approaches seems to be the only effective treatment to revert Rett phenotype, and some *in vitro* and *in vivo* studies using RTT mouse models have been performed in the last years. This project is a needed step in order to develop a treatment for human RTT patients, because it shows the possibility to revert the Rett-like phenotype through exogenous delivery of MeCP2 *via* AAV9 recombinant vectors in RTT mouse models. As *MECP2*-null mouse models' phenotype mimics with high accuracy the Rett patients' features, it is essential to develop great gene therapy procedures with low toxicity and improved survival using mouse models in order to create a treatment for the Rett syndrome affecting humans.

EXPERIMENT DESIGN

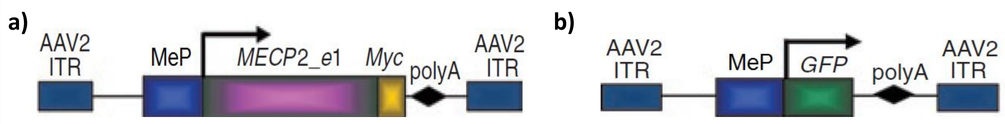


Figure 1. Vector design. The AAV vectors used in this study are AAV2 ITR-flanked genomes packaged into AAV9 serotype capsids with a truncated 229bp murine *MECP2* promoter (MeP.) **a)** AAV9/*MECP2* construct: it uses the human *MECP2* coding region (*MECP2_e1* mRNA isoform) with a C-terminal Myc epitope tag and the bovine growth hormone polyadenylation signal. **b)** AAV9/green fluorescent protein (*GFP*) using the SV40 polyadenylation signal.

Animals

3 groups (*n=13*):

- WT not treated (control)
- *Mecp2*$^{tm1.1/Bird}$ treated with AAV9-*MECP2* (study group)
- *Mecp2*$^{tm1.1/Bird}$ treated with AAV9-*GFP* (non-therapeutic control)

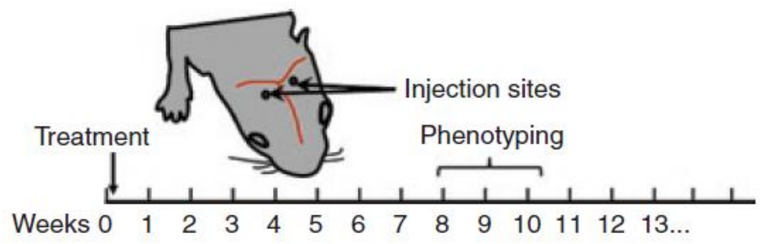


Figure 2. Experimental plan: injection and phenotyping start. Mice receive at P0-2 direct bilateral injection of virus into the neurophil (4'8x10^{10}vg/mouse). Phenotyping tests are carried out at w8-10.

RESULTS

- Neonatal CNS injection of AAV9/*MECP2* results in widespread brain expression of exogenous administrated MeCP2.
- AAV9/*MECP2* infection recapitulates MeCP2 expression in CNS cells from *MECP2*-null mice

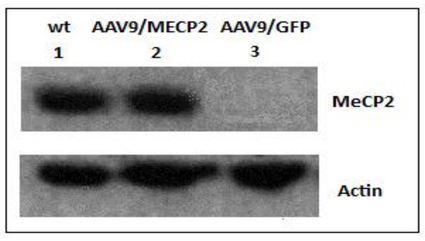


Figure 3. Detection of MeCP2 protein in wt mice and AAV9/*MECP2*-treated null mice but not in AAV9/*GFP*-treated null mice by western blot using actin as control.

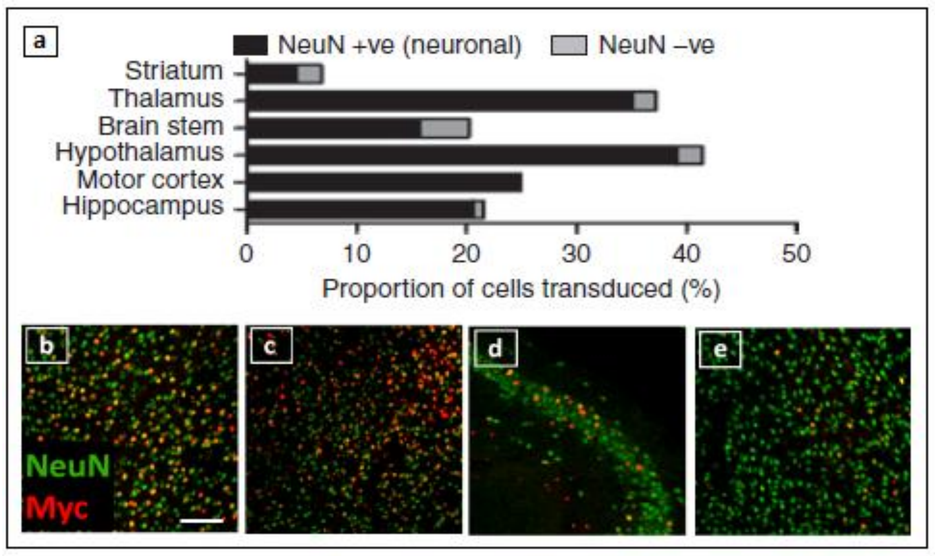


Figure 4 a) Quantification of transduction efficiency (as a proportion of DAPI-stained nuclei). **b-e)** Infected cells (Myc), Neurons (NeuN) and % of infected cells (overlapping dots).

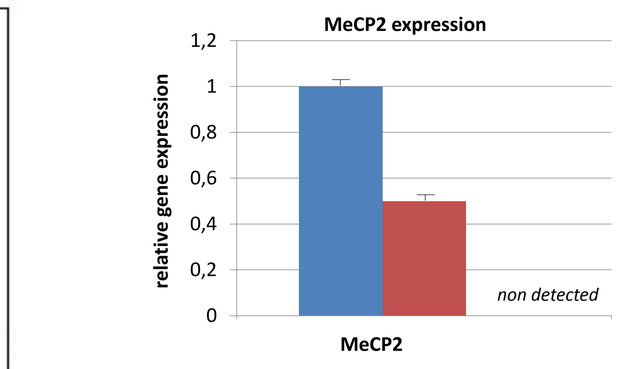


Figure 5. MeCP2 relative expression in brain samples from mice of each group. AAV9/*GFP*-treated null mice show no expression of MeCP2, whereas AAV9/*MECP2*-treated mice recapitulate the endogenous expression up to 50%.

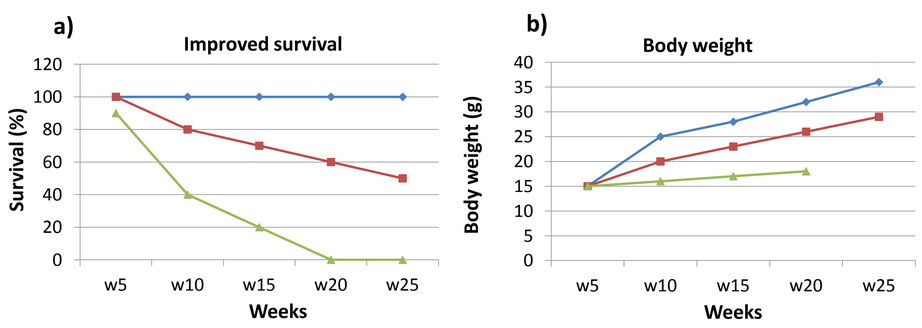
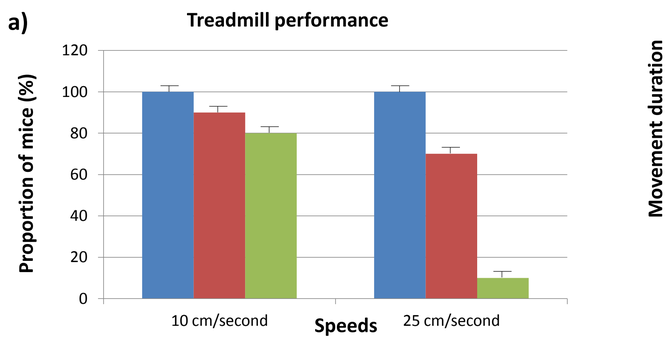
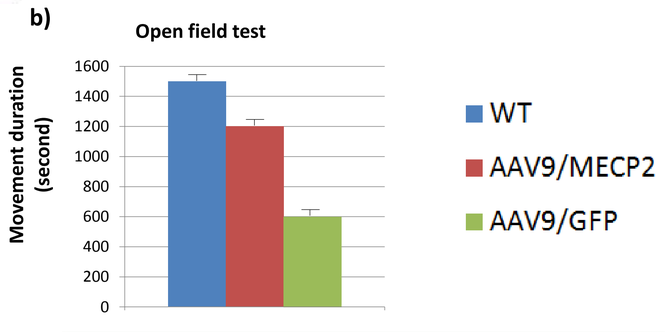


Figure 6. a) Improved survival of *MECP2*-null mice following AAV9/*MECP2* infection but not following AAV9/*GFP* infection. **b)** Improving of body weight in mice from each group.





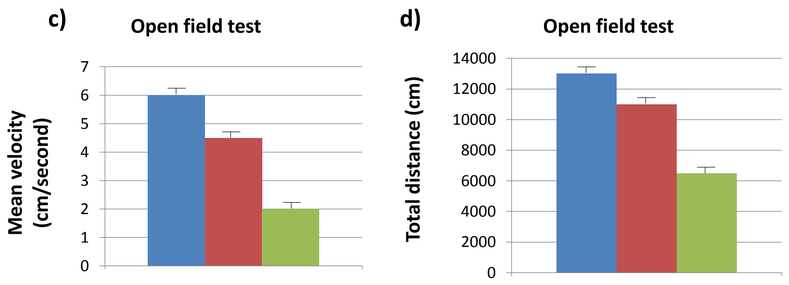


Figure 8. a) Treadmill performance at two different speeds (10cm/s and 25cm/s) performed by mice from every group. **b-d)** Open field duration measurements (movement duration, mean velocity and total distance).

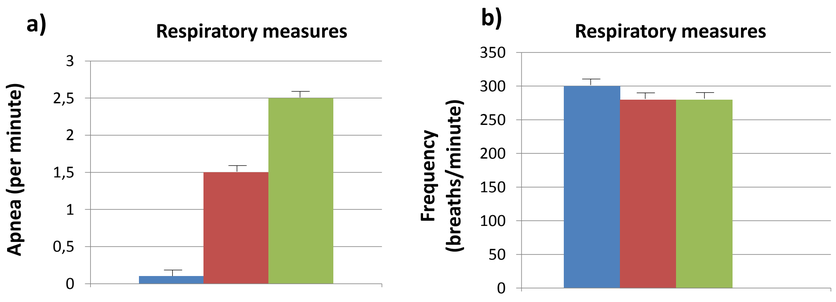


Figure 7. Respiratory measures. **a)** apnea per minute. **b)** breathing frequency (measured as breaths/minute).

Phenotypic reversion

- AAV9-mediated neonatal delivery of *MECP2* improves **survival** and **body growth** in *MECP2*-null mice.
- Restored normal **respiratory phenotype** in *MECP2*-null mice following AAV9-mediated neonatal delivery of *MECP2.*
- AAV9-mediated neonatal delivery of *MECP2* improves **motor phenotype** in *MECP2*-null mice.

DISCUSSION

This work shows the reversion of the Rett syndrome phenotype of *MECP2*-knockout mice following the exogenous delivery of *MECP2* through direct brain administration of AAV9/*MECP2* vector. A long-term expression of the exogenous protein is achieved and its expression pattern is similar to the endogenous one. Moreover, survival and body weight form *MECP2*-null mice are improved and phenotypic motor and respiratory abnormalities reverted.

REFERENCES

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