Preclinical Studies of Gene Therapy for the Rett Syndrome Treatment

Núria Tubau Juni
Degree in Biomedical Sciences. Universitat Autònoma de Barcelona

Introduction

Rett syndrome (RTT) is a X- dominant neurodevelopment disorder that affects females almost exclusively and the prevalence is approximately 1 in 10.000 live female births. About 90% of classical RTT cases are caused by mutations in Methyl-CpG binding protein 2 (*MECP2*) gene located in the locus Xq28. Patients with classic form seem to develop normally until 6-18 months old and then they start a regression of acquired activities, such as voluntary use of hands, lose speech, but also develop microcephaly, stereotypic hand movements, respiratory irregularities, and autism behaviour.

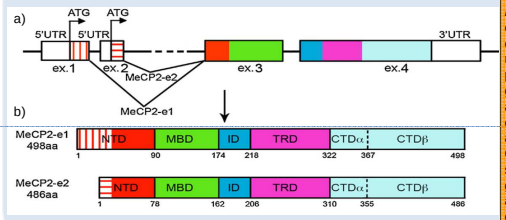
The aims of this study are to present a view of Rett syndrome's research and to expose the preclinical gene therapy trials published, in order to discuss the eficiency of this therapy and its possible aplication in humans.

Materials and Methods

- Search in Pubmed Database, using terms, such as Rett syndrome, gene therapy, MeCP2 and MeCP2-null animals. Papers were selected according to the year published and the importance of their results.
- Contact to Judith Armstrong, PhD.
- Visit patients webpages.

Results

MeCP2 is critical for the neurodevelopment and the maintenance of mature neurons. The most important domains of MeCP2 are MBD and TRD, essential for doing its function.



NTD: N-terminal domain; MBD: Methyl binding domain; ID: intervening domain; TRD: transcriptional repressor domain.; CTD: C-terminal domain

Figure 1. a) A diagram of *MECP2* gene, that contains 4 exons. In colours there is the representation of each protein domain in the gene sequence. *MECP2* also contain 2 start codons that leads to an alternative splicing. b) Owing to alternative splicing two isoforms are generated: MeCP2-e1 and MeCP2-e2. MeCP2-e1 is more abundant in brain [1].

Animal model	Generation	Phenotype
MeCP2$^{tm1.1\ Bird}$ (Mecp2-null mice)	Deletion of exons 3 and 4 of *Mecp2* by Cre system.	Normal until 6 weeks of age. After that, mutant mice developed motor impairments, irregular breathing, decreased body and brain weight, reduced neuronal size and had a premature lethality. Heterozygous females mice develop similar features but take longer to achieve it.
MeCP2$^{tm1.1\ Jae}$ (Mecp2-null mice)	Deletion of exon 3 of *Mecp2* by Cre system.	
MeCP2^{308}-mice (MeCP2-null mice)	Generation of a truncated *Mecp2* by the introduction of a premature STOP codon.	Male mutant mice exhibited RTT-like phenotype; in contrast, heterozygous females develop a milder phenotype.
MeCP2 disruption in Rhesus and Cynomologus monkeys	TALENT-mediated mutagenesis into exon 3 of *Mecp2*.	Male monkeys die in midgestation. One heterozygous female survive and was apparently normal at 4 months after birth.
MeCP2$^{Q63*/Q63*}$ (MeCP2-null zebrafish)	Production of a non-sense mutation in *Mecp2* that leads to a truncated protein at position 6.	Weak phenotype with no alterations apart from a shorter lifespan and some motor impairment.

There are three primary studies demonstrating the possible delivery of MeCP2 into the brain cells of *Mecp2-deficiency* mice *("in vitro"* and "in vivo")

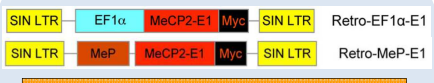


Figure 2. Retrovirues constructs use to transfect Neuronal stem cells (NSC) [2].

NSC derived from adult *Mecp2*$^{tm1.1Bird}$ - mouse

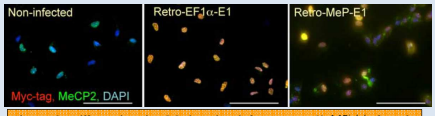


Figure 3. Differentiated NSC (14 days) from *Mecp2*$^{tm1.1Bird+/-}$ female mouse. Non-infected cells show mosaic *Mecp2* (endogenous) expression. Cells transfected (Retro-EF1α-E1 and Retro-MeP-E1) express exogenous MeCP2-E1 (Myc-tag). [2].

In comparison to control NSC, infected NSC (Retro-EF1α-E1) exhibit longer primary dendrites, occasionally secondary dendrites and neuronal networks began to be formed.

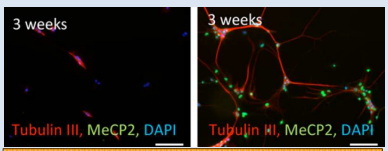


Figure 4. NSC from *Mecp2*$^{tm1.1Bird+/-}$ mice were differentiated for 3 weeks. For dendrite maturation analysis, NSC were stained for MeCP2 (green), DAPI (blue) and neuronal marker Tubulin III (red) [2].

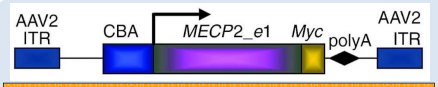
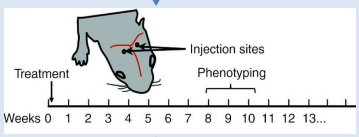
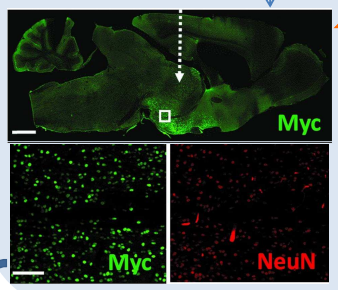


Figure 5. AVV9/CBA construct use to infect *Mecp2*$^{tm1.1Bird}$ male mice [3].

Figure 6. Neonatal male mice were bilateral injected into brain [3].





Survival
Motor phenotype
Nuclear volume

Figure 7. Widespread exogenous expression of MeCP2 through the brain. White arrow indicates the injection site. Proportion of Myc relative to NeuN population [3].

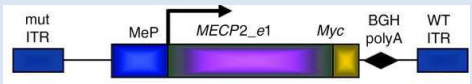


Figure 8. AVV9/MeP construct use to infect *Mecp2*$^{tm1.1Bird}$ male mice [3].

Introduced by intravenous delivery into four - five week-old *Mecp2*$^{tm1.1Bird}$ male mice

In intravenous delivery in juvenile male mice, the proportion of cells transduced was lower and the benefit is more limited.

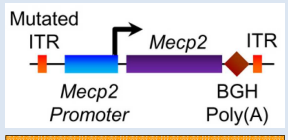


Figure 9. scAVV9/Mecp2 construct use to infect *Mecp2*$^{tm1.1Bird}$ mice [4].

Intravenous delivery into 4-6 weeks-old mice

scAAV9/MeCP2 injection resulted in a MeCP2 expression similar to endogenous levels throughout the brain.

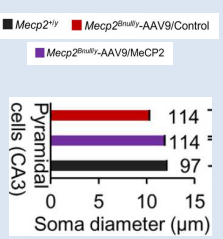


Figure 10. Average soma diameters of pyramidal cells (CA3) of MeCP2 positive, negative and wild-type mice [4].

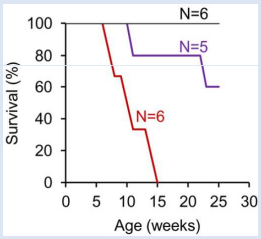


Figure 11. Survival percentage of MeCP2 positive, negative and wild-type mice [4].

The results in behaviour and motor test for these female injected mice were similar as wild-type mice. However, alterations in respiration were the only point that was not clear, since some injected females present alterations.

Conclusions

- Administration of *MECP2* through an AAV9 construct into *Mecp2-dificient* mice improves the phenotype and prolongs their lifespan.
- Neurons showed in transfected animals exhibit wild-type size, density and dendritic branch.
- Positive results suggest that gene therapy for RTT-patients is a challenge that could be achieved in the future. However, the investigation has to continue and new studies with big animals and trying new constructs, in order to accurate the therapy, have to be done.

References

1. Bedogni, F. *et al.* Rett syndrome and the urge of novel approaches to study MeCP2 functions and mechanisms of action. *Neurosci. Biobehav. Rev.* 1–15 (2014). doi:10.1016/j.neubiorev.2014.01.011
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3. Gadalla, K. K. E. *et al.* Europe PMC Funders Group Improved Survival and Reduced Phenotypic Severity Following AAV9 / MECP2 Gene Transfer to Neonatal and Juvenile Male Mecp2 Knockout Mice. **21,** 18–30 (2013).
4. Garg, S. K. *et al.* Systemic delivery of MeCP2 rescues behavioral and cellular deficits in female mouse models of Rett syndrome. *J. Neurosci.* **33,** 13612–20 (2013).