Rett Syndrome Gene Therapy: Understanding the Published Data

Authored by: Steve Kaminsky, PhD and Janice Ascano, PhD

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We have been asked by many families over the last two months to help them understand the status of gene therapy research for Rett syndrome. Below we summarize our review of the five published papers on gene therapy. It is important for all readers to know that this is our summary of the papers, and we apologize to the authors if we have over simplified their pioneering studies. Our goal is to condense the findings so that a general audience will have a fundamental understanding of the work to date.

What you need to know before reading the papers. In each of these five papers, male mice with mutations in the *Mecp2* gene were the animal model of choice. Only one paper extended their gene therapy study into the medically relevant female heterozygous mice with mutation in *Mecp2*. The importance of this distinction will be discussed in detail. First let us explain that MECP2 is the human gene, *Mecp2* is the mouse gene, and MeCP2 is the protein.

Male and female mice with mutations in *Mecp2* are very different in regard to their biology and their associated human conditions. Rett syndrome is a clinical diagnosis and very few males meet the clinical diagnostic features of Rett syndrome. In males, every cell in their body is affected by their mutation in *MECP2*. In females which are described as “heterozygous”, approximately half of the cells are normal, while the other half have the challenges of a mutated *MECP2* gene. In short, the genetics in Rett syndrome are complicated. The therapeutic intervention for boys with mutations in *MECP2* and girls with Rett syndrome will have to be investigated independently for safety reasons because of the fundamental difference in the cellular makeup in boys and girls with *MECP2* mutations.

Another point all readers should understand is that the virus used in these studies to deliver the *MECP2* gene is the adeno-associated virus (AAV). AAVs are the virus of choice for gene therapy because they can express the gene they’re carrying for a long time and do not cause disease or immune reactions in the host they are infecting. A particular AAV called AAV9 is of great interest for Rett syndrome gene therapy because it has been shown to cross the blood brain barrier after an intravenous (I.V.) injection and enter brain cells.

Summary of the Results

All five papers investigated the delivery of a normal copy of *MECP2* to male mice lacking a complete copy of *Mecp2* gene using an adeno-associated virus (AAV). Only one paper extended their study (Garg et. al.) to deliver a normal copy of *Mecp2* to a female model of Rett syndrome. A Summary Table of the experiments can be seen below. Each paper illustrated that they could deliver and express the normal *MECP2* in various regions of the body in their mouse models. The routes of delivery were either intravenous (I.V.) or directly into the brain, and each paper describes the challenges of these routes of delivery. The I.V. route for the most part showed that the liver often had a large uptake of the virus leading to liver toxicity due to expression of *MECP2* from the virus. This was a major concern that led up to the two 2017 papers, where the researchers concentrated on a direct injection of the virus into the brain. While direct brain injection showed that there was more expression in the brain than the liver, this route of delivery in humans is not without its challenges.

All five studies showed that they could extend the life of the male mice with *Mecp2* mutations, but never as long as their normal littermates. In addition, the effects on the scale of the severity of Rett syndrome were not changed or minimally changed in many of the experiments.

Lastly these papers clearly demonstrated the importance of the regulatory DNA elements that are inserted along with the normal *MECP2* gene. The length of the regulatory DNA elements is important to control the number of copies of *MECP2* produced, which affects the level of gene expression. Optimizing the length of the regulatory DNA enhanced the expression of *MECP2*, and thus resulted in improvement in survival and slightly improved the severity scale of the mutant male mice.
Summary Table of 5 Gene Therapy Published Papers in mice with Mecp2 mutations

<table>
<thead>
<tr>
<th>Paper</th>
<th>AAV used</th>
<th>Gender</th>
<th>Age</th>
<th>Route of delivery</th>
<th>Life span</th>
<th>Body weight</th>
<th>Severity scale</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gadalla et al. 2013</td>
<td>CBA-Mecp2</td>
<td>Males</td>
<td>0-3 days</td>
<td>Directly into the brain</td>
<td>from 9.3 to 16.6 weeks</td>
<td>no improvement</td>
<td>no improvement</td>
<td>not reported</td>
</tr>
<tr>
<td></td>
<td>MeP-Mecp2 V1</td>
<td>Males</td>
<td>4-5 weeks</td>
<td>Intravenous</td>
<td>from 10.6 to 15.4 weeks</td>
<td>no improvement</td>
<td>not studied</td>
<td>liver toxicity</td>
</tr>
<tr>
<td>Garg et al. 2013</td>
<td>MeP-Mecp2 GM</td>
<td>Males</td>
<td>4 weeks</td>
<td>Intravenous</td>
<td>from 10 to ~20 weeks</td>
<td>no improvement</td>
<td>no improvement</td>
<td>not reported</td>
</tr>
<tr>
<td>Matagne et al. 2017</td>
<td>MeP-Mecp2 JCR</td>
<td>Males</td>
<td>4 weeks</td>
<td>Intravenous</td>
<td>from 8 to 14.1 weeks</td>
<td>improvement</td>
<td>no improvement</td>
<td>not reported</td>
</tr>
<tr>
<td>Sinnett et al. 2017</td>
<td>MeP-Mecp2 V1 two doses</td>
<td>Males</td>
<td>4-5 weeks</td>
<td>Directly into the brain</td>
<td>from 8.1 to 12 weeks</td>
<td>no improvement</td>
<td>no improvement</td>
<td>liver toxicity</td>
</tr>
<tr>
<td></td>
<td>MeP-Mecp2 V1 two doses</td>
<td>Males</td>
<td>4-5 weeks</td>
<td>Directly into the brain</td>
<td>shortened lifespan</td>
<td>no improvement</td>
<td>no improvement</td>
<td>liver expression</td>
</tr>
<tr>
<td></td>
<td>MeP-Mecp2 V2 three doses</td>
<td>Males</td>
<td>4-5 weeks</td>
<td>Directly into the brain</td>
<td>from 8.1 to 11.6 weeks</td>
<td>slight improvement</td>
<td>no improvement</td>
<td>no liver expression</td>
</tr>
<tr>
<td>Gadalla et al. 2017</td>
<td>MeP-Mecp2 V1 three doses</td>
<td>Males</td>
<td>4-5 weeks</td>
<td>Intravenous</td>
<td>low dose- no change mid dose - from 11.6 to 27.1 wks high dose shortened lifespan</td>
<td>slight improvement</td>
<td>no improvement</td>
<td>liver toxicity</td>
</tr>
<tr>
<td></td>
<td>MeP-Mecp2 V1</td>
<td>Males</td>
<td>4-5 weeks</td>
<td>Intravenous</td>
<td>from 20.3 to 38.3 weeks</td>
<td>slight improvement</td>
<td>no improvement</td>
<td>not discussed</td>
</tr>
<tr>
<td></td>
<td>MeP-Mecp2 V2</td>
<td>Males</td>
<td>4-5 weeks</td>
<td>Intravenous</td>
<td>V1 from 11.6 to 27.1 weeks V2 from 11.6 to 29.9 weeks</td>
<td>slight improvement</td>
<td>no improvement</td>
<td>V2 does not have liver expression</td>
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<tr>
<td></td>
<td>MeP-Mecp2 V2</td>
<td>Males</td>
<td>0-3 days</td>
<td>Directly into the brain</td>
<td>from 12.4 to 38.6 weeks</td>
<td>no improvement</td>
<td>slight improvement</td>
<td>V2 does not have liver expression</td>
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</table>

For more detail on the five papers, please click on the links below:

Conclusions

These five studies each produce important contributions to gene therapy for Rett syndrome and provide the first steps toward a proof of concept that gene therapy is a viable treatment option to pursue. However, there are still many challenges in front of us.

In 2012, a special publication came out from the Rett syndrome research community published in Disease Models and Mechanism (Katz et al). One of the recommendations in this article was “To evaluate the robustness and generality of the original findings, we suggest validating promising treatment outcomes in more than one RTT mouse model/strain. Moreover, validating findings in female heterozygous Mecp2 mice is imperative.”

Only one of these gene therapy papers studied heterozygous female mice (Garg et al) and the I.V. administration led to a decrease in the severity of the phenotype (the visible characteristics produced by a gene). These authors pointed out this was a good first study that should lead to further studies evaluating the AAV for maximum brain uptake, the best mode of delivery, and best regulatory elements to control the expression of MECP2 in the female cellular environment. It is concerning that no other paper published data in heterozygous female mice.

We believe that validating findings in female mice must take place, especially in light of the Gadalla paper (2017) which showed that the second version of the virus was used to deliver MECP2 was detrimental to the health of the normal male mice. These are concerning results in regard to how this treatment might affect normal cells in the female heterozygous mice. It is critical to understand that girls with Rett syndrome have a normal MECP2 gene in half of their cells. Virus uptake in normal cells could push the girls into a condition of over expressing MeCP2 which could lead to a “MeCP2 duplication phenomenon”, a related MECP2 disorder that results from a duplication of the MECP2 gene. It is well agreed that MECP2 is sensitive to gene dosage, where too little or too much results in a multitude of problems.

In addition, we believe an independent laboratory (or a contract research organization) should conduct the replication study once a safe AAV containing MECP2 virus is identified in female mice. The replication study should attempt to duplicate exactly the original experimental design or modify the procedures (e.g. using a different Rett syndrome mouse model/strain) to evaluate the reproducibility of the findings. Why? Because
we’ve seen cases before where exciting results in a potential treatment (like bone marrow transplantation) were not reproduced in multiple labs after the original publication. Clinical trials are costly, and more importantly, drugs and biologics need rigorous, independent testing before being administered to a patient population.

Besides testing the most optimal AAV containing MECP2 virus in the medically relevant female mouse model, we have to recognize that there are many challenges to face as we try to map out the road of gene therapy.

- We need to better define the route of delivery.
  - I.V. is a systemic route but has off target effects in other organs, and direct delivery to the brain will have real complications when administering the virus to a human patient.
- We need to better define the actual vector to be used in gene therapy.
  - It is clear that the choice of regulatory DNA has some impact on MECP2 expression. However, we have seen that the current vector can detrimentally affect normal cells. Furthermore, the only experiment that was performed in the female mouse used the mouse MeCP2 gene, which is not a form that would be used in humans.
- We need to determine the right dosage in large animal primates.
  - The 2017 Sinnett paper pointed out, “Given the dosing limits determined in mice and the known side effects associated with overdosing, dose-ranging studies in large animals that are carefully monitored for side effects would be warranted before human translation should be considered.”

Future investigations in gene therapy will have to address pre-clinical studies including Good Laboratory Practices (GLP) and conduct sufficient animal safety and toxicity experiments to support an Investigational New Drug application. When one reads the Food and Drug Administration’s guidelines for clinical trials in cell and gene therapy, it is evident that there is still a lot in front of the research community as we all prepare for these types of trials in Rett syndrome.