Examining the Role of Arginase1 Overexpression in an Animal Model of Tauopathy

Shannon Varughese[1], Andrii Kovalenko[1], Kevin Ratnasamy[1], Jerry Hunt Jr[1], Sarah N. Fontaine, PhD[2], Leslie A. Sandusky, PhD[1], Chad Dickey, PhD[2], Daniel C. Lee, PhD[1]

[1]Byrd Alzheimer's Institute, University of South Florida, Tampa, FL 33613: College of Pharmacy, Department of Pharmaceutical Sciences, University of South Florida, Tampa

[2]Byrd Alzheimer's Institute, University of South Florida, Tampa, FL 33613: College of Medicine, Department of Molecular Medicine, University of South Florida, Tampa

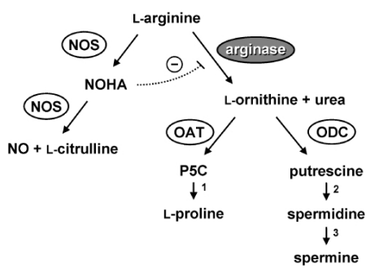
Background

Hyperphosphorylation of tau is associated with cognitive impairment and decline in memory processes, neuronal loss, and development of neurofibrillary tangles and plaques in the brain. These factors are characteristic of neurological disorders such as Alzheimer's Disease (AD) which are becoming increasingly widespread worldwide.

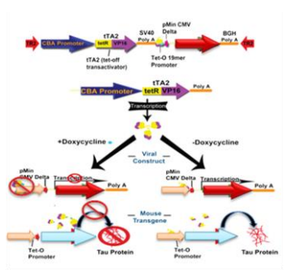
Polyamines are of interest when studying AD as these metabolites may have neuroprotective roles. An essential pathway that leads to production of polyamines is the L-arginine pathway, which can go two ways. Metabolism of L-arginine by nitric oxide synthase (NOS) produces citrulline and nitric oxide, which are believed to contribute to increased oxidative stress in the brain. Metabolism of L-arginine by arginase1 leads to production of polyamines. Therefore, metabolism of L-arginine by arginase1 is of interest.

In order to further investigate the role of arginase1 and its potential role on tauopathy, we utilized the transgenic tetO MAPT P301L model. The model described here is highly unique because it allowed for regional expression of tau in the hippocampus that concurrently expressed neuropathology. Moreover, it was driven by a tetracycline transactivator protein (tTa2) while utilizing a novel technique for potential treatment through use of viral mediated gene therapy (adeno-associated virus serotype 9; AAV9). The tTa2 protein would therefore drive expression of tau and either treatment GFP or arginase1. We postulate that arginase1 may be a potential therapeutic for the treatment of tauopathy and neuropathology.

Metabolism of L-arginine



Viral Construct:

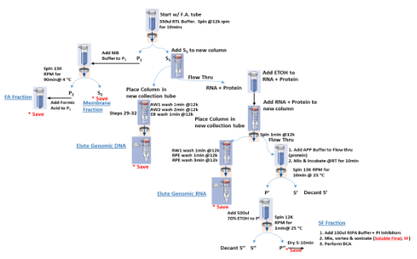


Experimental Design

The design of this study involved six different groups: (1) non-transgenic given AAV9-Empty Capsid, (2) non-transgenic given AAV9-tTA-GFP, (3) non-transgenic given AAV9-tTA-ARG, (4) TetO MAPT P301L given AAV9-Empty Capsid, (5) TetO MAPT P301L given AAV9-tTA-GFP, and (6) TetO MAPT P301L given AAV9-tTA-ARG1. All mice were injected bilaterally in the hippocampus and allowed to incubate for a period of 14-weeks before tissue collection.

Group	Genotype	Treatment	N
1	nTg	AAV9-Empty C	5
2	nTg	AAV9-tTa-GFP	6
3	nTg	AAV9-tTa-ARG1	7
4	TetO (MAPT P301L)	AAV9-Empty C	5
5	TetO (MAPT P301L)	AAV9-tTa-GFP	8
6	TetO (MAPT P301L)	AAV9-tTa-ARG1	7

DNA/RNA/Protein Extraction: The AllPrep DNA/RNA/Protein kit was used to extract DNA, RNA, and 3 different protein fractions from the animal tissue. The soluble fraction of the protein was used for this study. A flow chart illustrating the methods involved in the AllPrep Kit is below.



Western Blotting Analysis: Equal amounts of protein from each sample was loaded onto 4-20% Tris-Glycine SDS-PAGE gels and transferred onto a 0.45 µm PVDF membrane. The membranes were blocked in 5% milk in TBS at room temperature for 30 minutes and then probed with different primary antibodies overnight. The membranes were then washed with 1X TBST (TBS, 0.07% Tween-20) 3 times, 10 minutes each. Afterwards, the membranes were incubated in secondary antibody for a period of 2 hours. Once again, the membranes were washed 3x, 10 minutes each with 1x TBST. Detection was done with Pierce ECL Western Blotting Substrate Reagents. The different antibodies probed for include 1:1000 dilutions of Total Tau H150 and Arginase1, a 1:3,000 dilution of GFP, and a 1:10,000 dilutions of Total Tau 5, Tau pser199/202.

Acknowledgements:

This work was supported by the Alzheimer's Association (MNIRDG)

Results

Figure 1: AAV9-tTa-GFP successfully induces GFP expression, AAV9-tTa-ARG1 induces ARG1 expression.

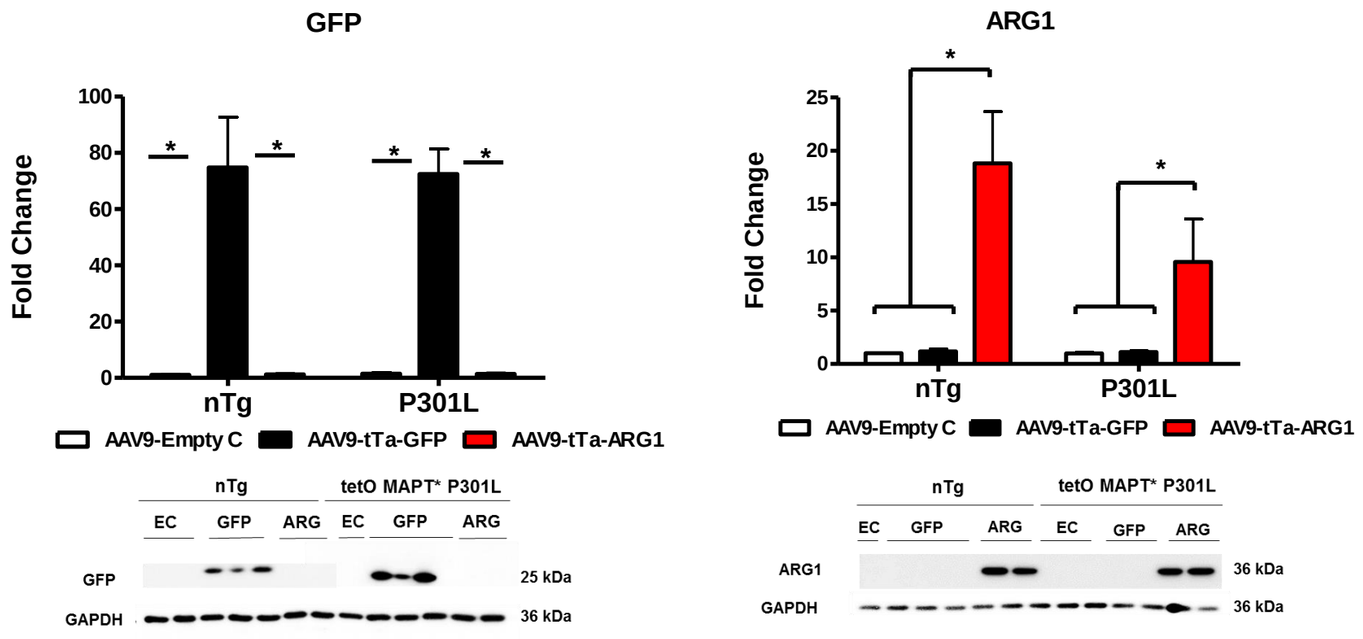


Figure 1: AAV9-tTA-GFP successfully induces GFP [A] expression in both nontransgenic (nTg) and transgenic (tetO MAPT P301L) mice. There is no significant difference between GFP expression amongst the two genotypes. Data was analyzed using a two-way ANOVA test and Fisher's PLSD, $p < 0.0001$.

Figure 2: AAV9-tTa-GFP overexpression induces tau, while AAV9-tTa-ARG1 reduces pathology.

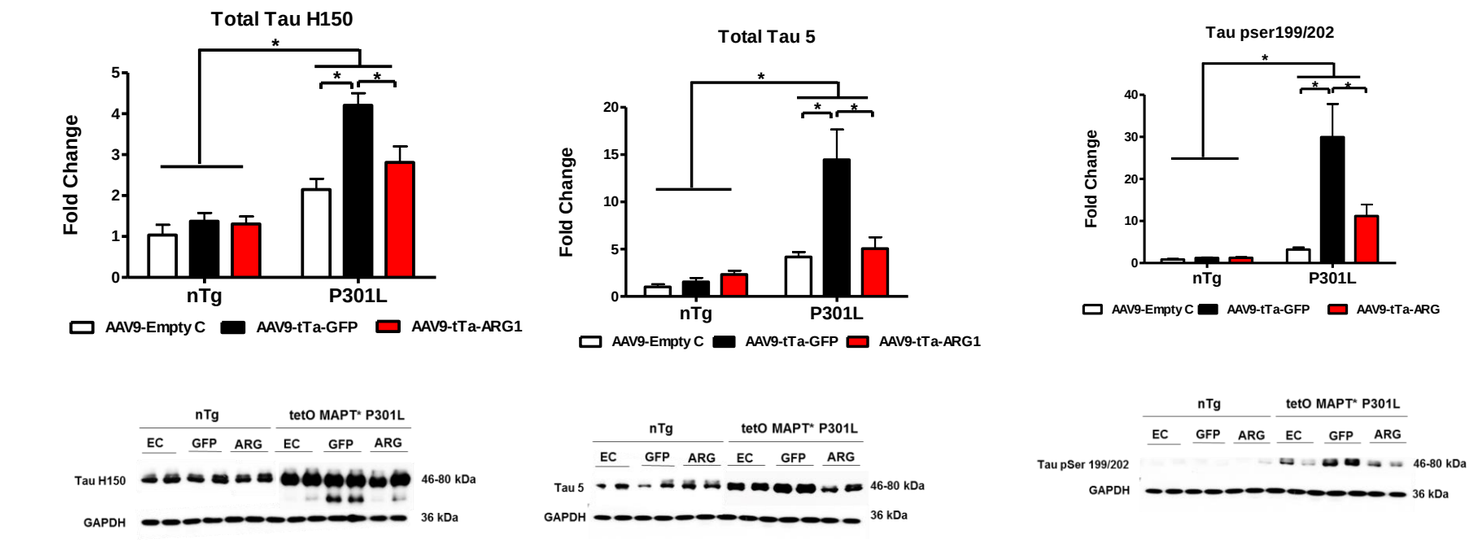


Figure 2: AAV9-tTa-ARG1 successfully induces Arginase1 [B] expression in both nTg and tetO MAPT P301L mice. There is no significant difference between arginase1 expression amongst the two genotypes. Data was analyzed using a two-way ANOVA test and Fisher's PLSD. $F(2,32)=4.907$, $p < 0.05$.

Figure 3: Increase in pmTOR suggests an impact on autophagy in AAV9-tTa-GFP injected transgenic mice.

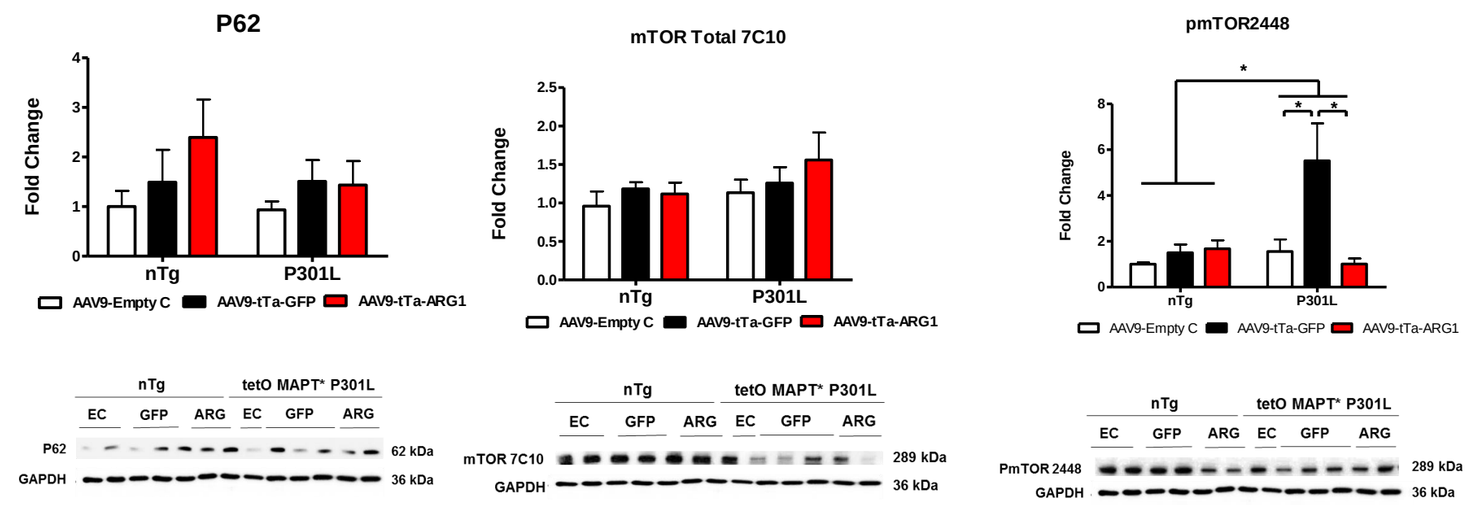


Figure 3: tetO MAPT P301L mice show significantly increased levels of total Tau H150 [A], total Tau5 [B], and phosphorylated Tau pSer199/202 [C] when compared to nTg mice. AAV9-tTa-ARG1 significantly reduces total Tau H150 [A], total Tau 5 [B], and Tau pSer199/202 [C] expression compared to AAV9-tTa-GFP treated tetO MAPT P301L mice. Total Tau H150, Tau 5, and Tau pSer199/202 were quantified using percent area to measure levels of the protein in the animals. Data was analyzed using a 2x2 Factorial ANOVA (Genotype x Treatment) and Fisher's PLSD. There were no significant differences amongst genotypes or treatments for P62 and total mTOR expression [D,E]. Data reveals a notable difference in expression of pmTOR[F] amongst treatment within transgenic mice; those injected with AAV9-tTa-GFP exhibit higher expression than those treated with ARG1 or Empty Capsid constructs within transgenic mice.

Conclusions & Future Directions

- Using the tetO MAPT*P301L model successfully allowed for regional expression of tau within the hippocampus.
- Using this model driven by the tetracycline transactivator protein (tTa2) allowed for concurrent expression of tau and the genes GFP and arginase1.
- Arginase1 overexpression driven by tTa2 was able to significantly decrease expression of total tau forms and phosphorylated tau in comparison to tTa2 driving GFP.
- Arginase1 may serve as a potential therapeutic gene of interest for treatment of tau neuropathology.