Route of Administration Investigation of a Novel AAV9GATM Construct in a AGAT Deficient Murine Model

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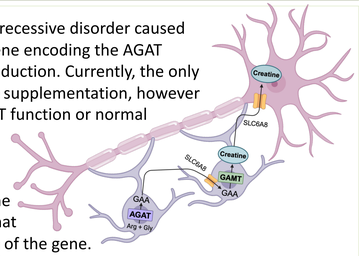
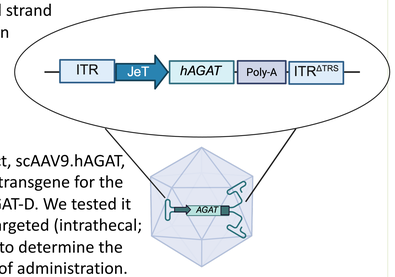
Background

AGAT-deficiency (AGAT-D) is an autosomal recessive disorder caused by loss of function mutations in the *GATM* gene encoding the AGAT protein, preventing endogenous creatine production. Currently, the only treatment for AGAT-D relies on daily creatine supplementation, however this is not curative and does not restore AGAT function or normal creatine levels in the brain, leaving many symptoms untreated.



Gene replacement therapy is defined by the delivery of a functioning transgene to cells that either lack or contain a dysfunctional version of the gene. This therapy is best suited for single gene deficiencies, such as AGAT-D. The transgene is typically encapsulated in a viral vector that has undergone recombinant engineering to remove the infectious viral genes. Adeno-associated vectors (AAV) are favoured due to their ability to transduce many cell types, low cytotoxicity, and sustained transgene expression. AAVs can be further engineered into self-complementary (scAAV) forms to eliminate the rate limiting step of second strand synthesis and produce the desired protein sooner. Serotype 9 (AAV9) has the unique ability to cross the blood brain barrier (BBB) for the treatment of neurological disorders.



Here we have created a novel construct, scAAV9.hAGAT, that encodes the human *GATM* (hAGAT) transgene for the peripheral and neuronal treatment of AGAT-D. We tested it through systemic (intravenous; IV) and targeted (intrathecal; IT) injection routes at comparable doses to determine the success of the vector and the best route of administration.

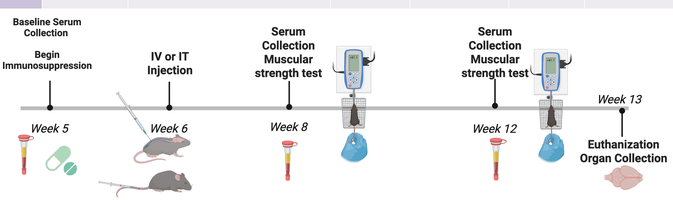
Hypothesis and Objectives

Gene replacement of *GATM* (AGAT) restores AGAT protein production and creatine levels in AGAT-D

1. Restore AGAT protein production in treated AGAT KO mice
2. Restore creatine production in the periphery and CNS in treated AGAT KO mice
3. Avoid GAA accumulation in the CNS from AGAT overexpression
4. Assess phenotypic benefits through weight gain and forelimb muscular strength test

Methods

Cohort	Genotype	Vector type	Injection method	Dosage (vg/mouse)	Mice (n)	Endpoint (weeks)
1	AGAT$^{+/-}$	Vehicle	IV/IT	0	8	13
2	AGAT$^{-/-}$	Vehicle	IV/IT	0	8	13
3	AGAT$^{-/-}$	scAAV9.hAGAT	IV	7.88x10^{11}	7	13
4	AGAT$^{-/-}$	scAAV9.hAGAT	IT	7.88x10^{10}	8	13



Results

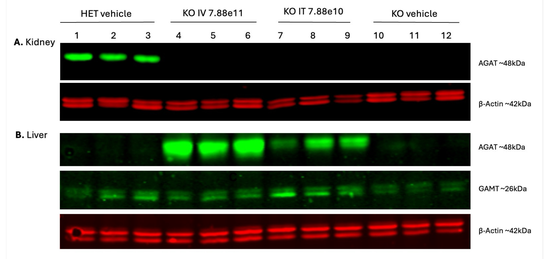


Figure 1. AGAT is primarily found in the kidneys (A), demonstrated by the heterozygote control samples. Protein production is restored in AGAT KO mice treated with scAAV9.hAGAT as seen in liver samples (B). IV treated mice demonstrated a significant increase in AGAT signal intensity as compared to the KO controls (C) (p<0.05).

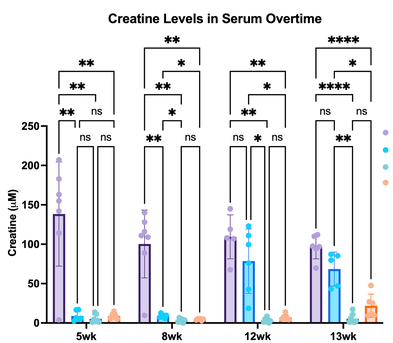


Figure 2. Serum creatine levels were significantly increased in IV treated AGAT KO mice compared to the vehicle treated counterparts as early as 2 weeks post injection (8wk time point) and continued to rise (p<0.05). No difference in serum creatine levels were observed in the IT treated mice.

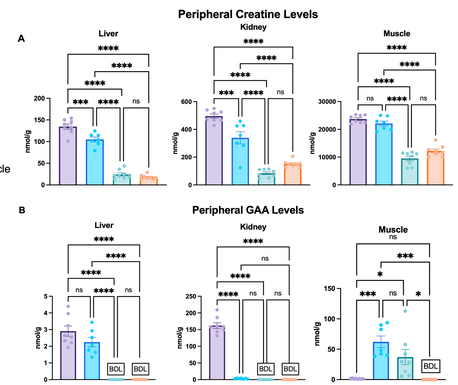


*Figure 3. **A.** Creatine levels were significantly increased in the periphery of the IV treated KO mice compared to the vehicle treated counterparts (p<0.0001). No increase was observed in the IT treated KO mice. **B**. Increased GAA levels were observed in the muscle of treated mice; however, this is not a concern as GAA is not myotoxic. BDL: below detectable limit*

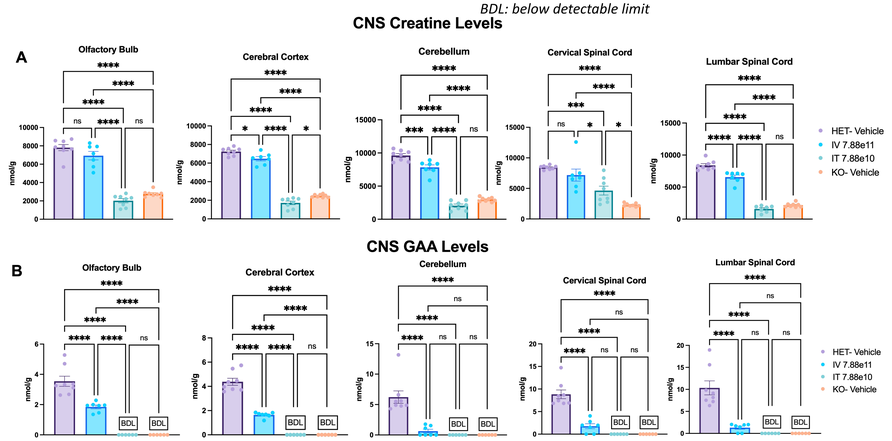


*Figure 4. **A.** Creatine levels were significantly increased in all areas of the CNS in the IV treated mice as compared to KO vehicle controls (p<0.0001). There was no difference in creatine levels observed in the majority of the IT treated KO mice, apart from the cervical spinal cord (p<0.05). **B.** Elevated GAA levels were not observed in any areas of the CNS of treated KO mice. BDL: below detectable limit*

Results

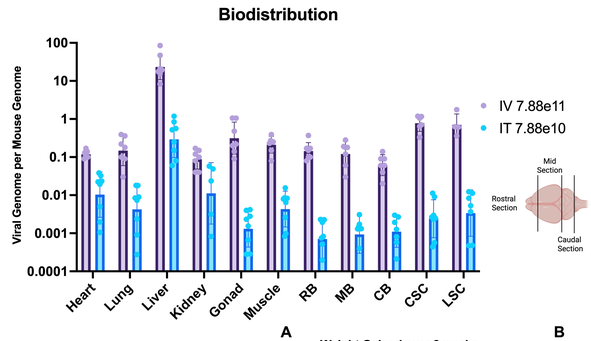


Figure 5. Viral genomes were present in all organ systems of the AGAT KO treated mice displaying the systemic reach of the vector and injection methods. The most copies were found in the liver as expected with appreciable copies detected in the CNS, demonstrating the vectors' ability to cross the BBB when delivered IV. The viral genomes were about 40-fold lower in the CNS of the IT treated mice, demonstrating that the dosage was too low to provide a comparable CNS effect.

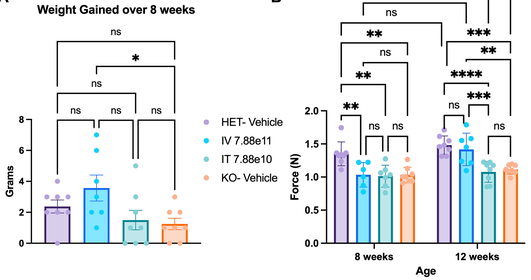


*Figure 6. Untreated AGAT KO mice have a reduced fat content and muscle atrophy. **A.** The IV treated KO mice had a significant weight increase as compared to the KO vehicle controls (p<0.05). **B.** At 8 weeks of age, all the KO cohorts had similar strength. The IV treated KO mice saw a significant increase in strength from the 8week to the 12week time point and were significantly stronger than the KO vehicle mice at 12 weeks of age (p<0.0001, p<0.01).*

Conclusion

The novel sc.AAV9.hAGAT construct is able to restore the biochemical and peripheral phenotype of AGAT-D in a murine model.

- ❖ AGAT protein production was restored in scAAV9.hAGAT treated mice.
- ❖ Serum creatine levels increased two weeks post scAAV9.hAGAT treatment and continued to rise overtime in the IV treated mice.
- ❖ Creatine production was restored throughout the body in the IV treated mice without concerning GAA accumulation.
- ❖ Biodistribution of the vector demonstrated systemic circulation, the vector's ability to cross the BBB, and that the dosage was too low to produce the necessary viral copies for the IT treated mice.
- ❖ The vector was able to elicit a phenotype restoration in the IV injected mice through increased weight gain and muscle strength.

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Association for Creatine Deficiencies

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