National Human Genome Research Institute

#404 Anc80 Mediates Hepatic Correction of Methylmalonyl-CoA Mutase Deficiency in Murine Models of Methymalonic Acidemia

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Abstract

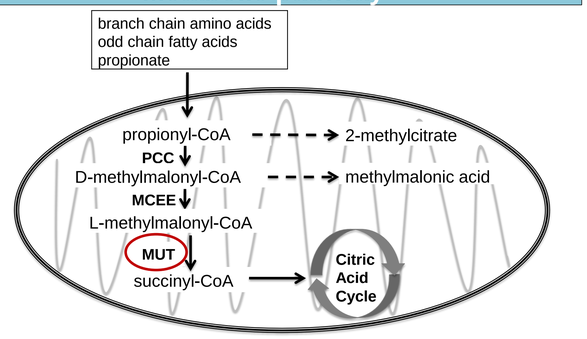
Other than dietary and cofactor therapy, no alternative to organ transplantation exists for patients with isolated methylmalonic acidemia (MMA), a common and severe organic acidemia most frequently caused by mutations in the enzyme methylmalonyl-CoA mutase (MUT). *Mut* knock-out (*Mut*$^{-/-}$) mice replicate the phenotype of the most severe form of MMA and perish in the immediate newborn period. The introduction of a germ line transgene configured to express *Mut* in the skeletal muscle of *Mut*$^{-/-}$ mice has allowed the generation of mice, *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$, that are rescued from lethality yet display severe biochemical perturbations, growth failure, and hepatopathy. *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice accurately mirror the severe childhood form of isolated MMA and provide a more physiologically relevant model to assay systemic gene therapy than neonatal *Mut*$^{-/-}$ pups. We have therefore used adult *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice to test the effects of systemic AAV gene therapy to mediate hepatic expression of MUT. We compared a canonical hepatotrophic AAV serotype 8 vector configured to express the human MUT gene under the control of the alpha-1 antitrypsin promoter (AAV8-hAAT-MUT) to the same vector transgene pseudotyped with the novel capsid, Anc80 (Anc80-hAAT-MUT). Anc80 is an in silico-designed synthetic capsid and a putative ancestor of natural AAV serotypes including AAV2, AAV8 and AAV9 with a reduced cross-reactivity with naturally occurring AAV serotypes.

Adult female *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice received 5x10^{12} GC/kg of either Anc80 or AAV8 vector (n=3 per group) delivered by retro-orbital injection. Plasma methylmalonic acid and methylcitrate concentrations and weight were measured before and post AAV gene therapy on day 12, 30 and 60. 1-^{13}C propionate oxidative capacity was measured on day 12 post AAV gene therapy. Both vectors induced a robust biochemical and clinical response by day 12. Plasma methylmalonic acid levels dropped from 985±86 uM to 173±28 uM for the Anc80 vector and from 1153±511 uM to 176±31 uM for the AAV8 vector, and were paralleled by substantial weight gain from 20.1±1.9g to 26.2±2.5g for the Anc80 vector and from 21.9±2.9 g to 24.3±2.8g for the AAV8 vector. A significant increase in the oxidative capacity for propionate (see figure) were observed on D12 post Anc80 or AAV8 gene therapy. The AAV treated animals maintained their weight and metabolic stability on D30 and 60, but showed no significant changes compared to the D12 time point. In addition, Anc80-hAAT-MUT vector was able to rescue the lethal phenotype in the neonatal *Mut*$^{-/-}$ mice model at dose as low as 1x10^{10} GC/pup. These studies show the functional equivalency of AAV8 and Anc80 vectors for the correction of hepatic *Mut* deficiency in mouse models of MMA, and demonstrate the utility of the *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice to rapidly assay vector efficacy. The addition of synthetic vaccine particles encapsulating rapamycin (SVP)-Rapamycin to Anco80 inhibited the formation of anti-Anc80 IgG antibodies, indicating the potential to re-administer Anc80 vectors. The ability to re-administer gene therapy to pediatric MMA patients may be critical for these patients, as transgene expression is expected to wane over time as the patents grow.

The findings support further investigation of Anc80-hAAT-MUT with the goal to develop an effective gene therapy that can be effective in patients with pre-existing antibodies to naturally occurring AAV serotypes and enable retreatment at a later date.

Background

Metabolic pathway

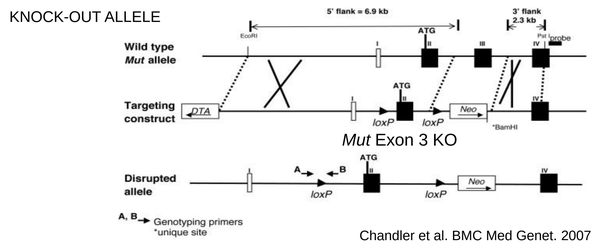


Methylmalonic Acidemia (MMA)

- MMA is an inherited disorder in which the body is unable to metabolize propionyl-CoA.
- Common organic acidemia with a prevalence of ~1:50,000 at birth.
- High mortality and morbidity in childhood.
- Characterized by metabolic instability, poor growth and massive accumulation of methylmalonic acid in the blood and urine
- Most patients have mutations in the methylmalonyl-CoA mutase (MUT) gene
- Elective liver transplantation used to treat some patients
- Gene therapy, systemic and liver-directed, in young patients has the potential to correct the major complications of the disease.
- However the ability to re-administer gene therapy as the children grow older is an important consideration.
- Currently vector re-administration is limited by the formation of neutralizing antibodies.

Murine Models of MMA

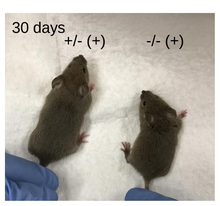
Neonatal Lethal Model: *Mut* $^{-/-}$



Chandler et al. BMC Med Genet. 2007

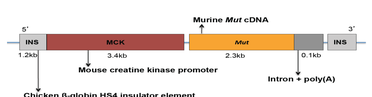
Hepatorenal cerebral: *Mut* $^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$

Severe MMA phenotype but rescued from lethality by a transgene that expresses Mut in the muscle



Muscle Specific *Mut* Rescue

Tg$^{INS\text{-}MCK\text{-}Mut}$ rescue construct



GROWTH RETARDATION

MCK TRANSGENE

Anc80

- In silico-designed synthetic AAV capsid
- A putative ancestor of natural AAV serotypes AAV2, AAV8 and AAV9
- A reduced cross-reactivity with naturally occurring AAV serotypes

SVP-Rapamycin

- Biodegradable synthetic vaccine particles encapsulating rapamycin, an mTOR inhibitor
- Mitigates immunogenicity of biologic drugs

Experimental design

Mut$^{-/-}$ neonatal

Anc80 or AAV8 intra-hepatic injection

Day of Life 1

Mut$^{-/-}$ mice

AAV:

Anc80L65-hAAT-synMUT4-RBG
AAV8-hAAT-synMUT4-RBG
ApoE = ApoE enhancer
hAAT= alpha-1 antitrypsin promoter

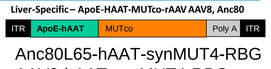
Dose: 1E10 GC/pup

Mut$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$

Adult *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice

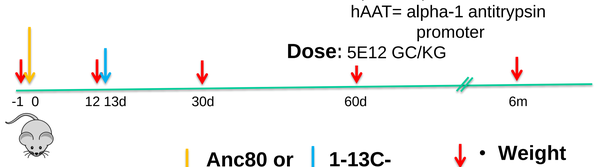
Anc80 or AAV8 retro-orbital injection

AAV:



Anc80L65-hAAT-synMUT4-RBG
AAV8-hAAT-synMUT4-RBG
ApoE = ApoE enhancer
hAAT= alpha-1 antitrypsin promoter

Dose: 5E12 GC/KG



Anc80 or AAV8 → **1-13C-Propionate oxidation** → ↓ Weight, ↓ MMA, ↓ MC

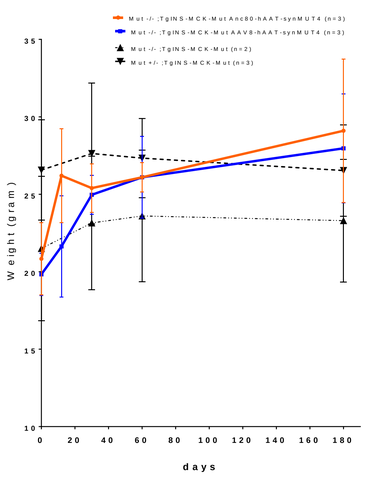
Results

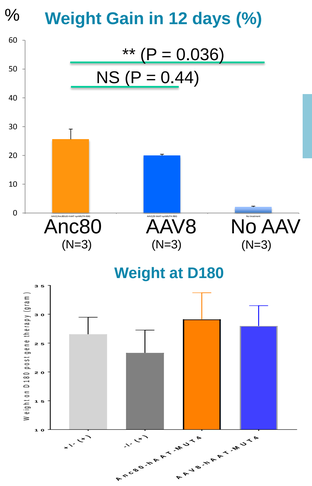
Mut$^{-/-}$ neonatal rescue

Vector	Number of treated mice (1E10 per pup)	Number of Mut -/- mice	Number of Mut -/- alive on Day 200
AAV2/8-hAAT-synMUT4-RBG	8	2	1
AAV2/Anc80L65-hAAT-synMUT4-RBG	11	1	1

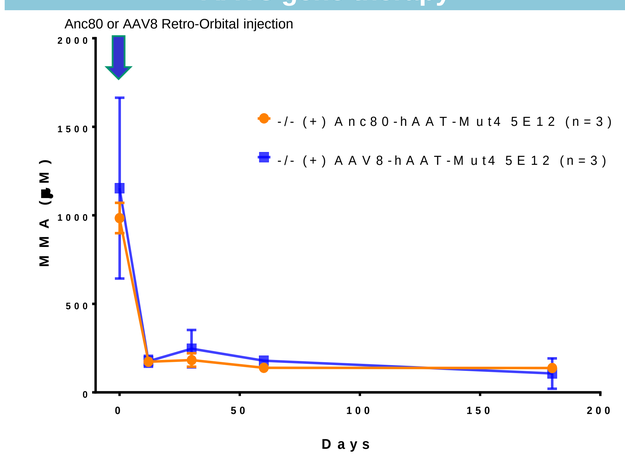
Anc80 or AAV8 vector rescued the lethal phenotype in the neonatal Mut-/- mice model at a dose as low as 1x1010 GC/pup

Weight Gain in *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ treated with Anc80 or AAV8-hAAT-Mut4

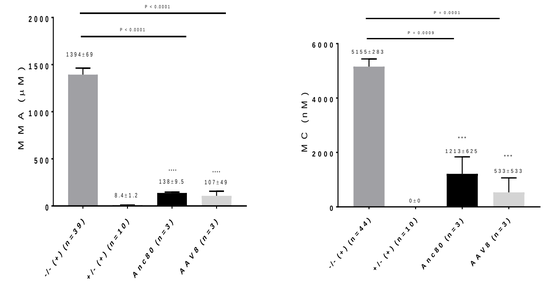




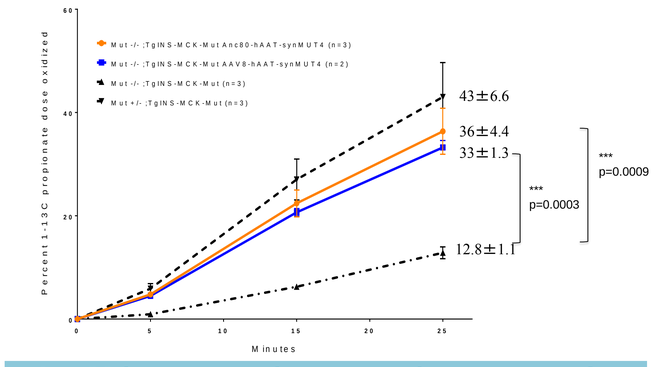
Plasma [MMA] levels decrease after Anc80 or AAV8 gene therapy



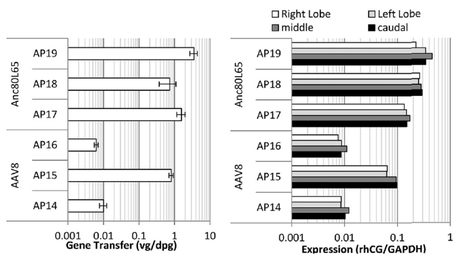
[MMA] and MC decrease at D180 in Anc80 or AAV8-hAAT-MUT treated mice



In vivo 1-^{13}C-Propionate oxidation increased in Anc80 or AAV8-hAAT-MUT treated mice



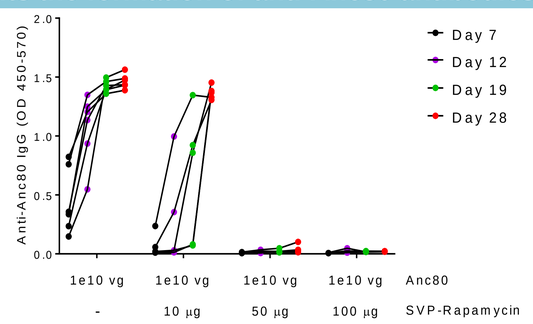
Anc80 is a potent hepatotropic gene transfer vector



AAV8 and Anc80L65 expressing Rhesus chorionic-gonadotropin (rhCG) was administered by saphenous vein injection at a dose of 1 X 10^{12} GCs/kg in Rhesus macaque. Viiral genome (vg) per diploid genome (dpg) and rhCG expressed was assessed

Zinn et al. Cell Reports. 2015

Addition of SVP-Rapamycin to Anc80 vector inhibits the formation of anti-Anc80 antibodies



C57Bl/6 mice (n=6/group) were administered Anc80 (1e10 vg/mouse) without or with 10, 50, or 100 μg SVP-Rapamycin at doses. Anti-Anc80 IgG are shown for individual animals in each cohort at various time points as indicated.

Conclusions

- These studies show the functional equivalency of AAV8 and Anc80 vectors for the correction of hepatic *Mut* deficiency in mouse models of MMA, and demonstrate the utility of the *Mut*$^{-/-}$;Tg$^{INS\text{-}MCK\text{-}Mut}$ mice to rapidly assay vector efficacy.
- Furthermore, the addition of SVP-Rapamycin to Anc80 was effective in inhibiting the antibody response to Anc80, suggesting the potential to re-administer Anc80 by avoiding the formation of neutralizing antibodies.
- The findings support further investigation of Anc80-hAAT-MUT with the goal to develop an effective gene therapy that can be effective in patients with pre-existing antibodies to naturally occurring rAAV serotypes and enable retreatment at a later date.

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