Background

Other than dietary and co-factor 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−/−; TgMCK-MUT, that are rescued from lethality yet display severe biochemical perturbations, growth failure, and hepatopathy. Mut−/−; TgMCK-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−/−; TgMCK-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 transgenic pseudotyped with the novel capsid, Anc80 (AAV8-AntA-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−/−; TgMCK-MUT mice received 5×1010 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. 125I pertechnate 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±286 µM to 175±56 µM for the Anc80 vector and from 1153±511 µM to 176±31 µM for the AAV8 vector, and were paralleled by substantial weight gain from 20±1.2±9.0 to 26±2.2±5.6 g for the Anc80 vector and from 21±9.2 g to 24±3.2±2.8 g for the AAV8 vector. A significant increase in the oxidative capacity for pertechnate (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 1×1010 GC/pup. These studies show the functional equivalency of AAVs and Anc80 vectors for the correction of hepatic MUT deficiency in mouse models of MMA, and demonstrate the utility of the Mut−/−; TgMCK-MUT mice to rapidly assay vector efficacy. The addition of synthetic vaccine particles encapsulating rapamycin (SVP-Rapamycin) to Anc80 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 patients 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.

Experimental design

**Mut−/− neonatal**

Anc80 or AAV8 intra-hepatic injection

**Mut−/−; TgMCK-MUT**

Anc80 or AAV8 retro-orbital injection

**Results**

**Mut−/− neonatal rescue**

- **Weight Gain in Mut−/−; TgMCK-MUT treated with Anc80 or AAV8-hAAT-MUT4**

  - Anc80 or AAV8 vector rescued the lethal phenotype in the neonatal Mut−/− mice model at a dose as low as 1×1010 GC/pup.

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

  - 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

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−/−; TgMCK-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 AAV serotypes and enable retreatment at a later date.