Long-term Rescue of a Neonatal Lethal Form of OTC Deficiency by Multiple Treatments with AAV Vectors of Different Serotypes

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Objective

Ornithine transcarbamylase deficiency (OTCD) accounts for nearly half of all inborn errors of the urea cycle. While the majority of patients have late-onset disease, those with severely deficient enzyme activity experience hyperammonemic crisis within the first 30 days after birth, leading to irreversible cognitive deficits and 50% mortality. AAV-based gene therapy would provide an alternative to the limited treatment options currently available.

Previously, we developed a clinical candidate vector, an AAV2/8 based self-complementary vector expressing a codon-optimized human OTC gene (hOTCco) driven by a liver-specific TBG promoter. Robust and sustained correction of the OTCD biomarker orotic aciduria and clinical protection against an ammonia challenge were achieved by a single tail vein injection of the vector in adult spfash mice (partial OTCD). In the current study, we used this vector to rescue mice with severe neonatal onset OTCD.

Methods

1. Vector: scAAV2/8.TBG.hOTCco and scAAV2/rh10.TBG.hOTCco (produced by the Penn Vector Core)

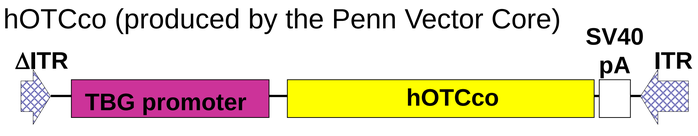


Figure 1. Diagram of scAAV.TBG.hOTCco

2. OTC-KO: A mouse model in which exons 2-3 of the OTC gene was replaced with a neomycin cassette. Affected male pups perish within 24 hours after birth.

3. Male pups were retrieved from timed mating dams on the due date and immediately after revival, they received AAV8-hOTC vector treatment via the temporal vein or direct intrahepatic injection. At the 4-weeks of age, rescued OTC-KO mice received a second vector administration (AAVrh10-hOTC) i.p. Rescued KO mice were monitored for body weight, plasma NH3 levels, and urine orotic acid levels.

Results

Untreated male OTC KO pups died within 24 hours after birth, a single temporal vein injection of $3x10^{10}$ GC of scAAV8-hOTCco immediately after birth successfully rescued the OTC KO pups and extended their life to 6 weeks. Efficacy was not maintained beyond 6 weeks, likely due to loss of vector genome during neonatal liver proliferation. To achieve long-term correction, a second dose of scAAVrh10-hOTCco was then delivered to 4-week-old rescued OTC-KO mice. Among 22 animals, 32% survived up to 18 months of age (end of the study), with plasma ammonia levels remaining in normal range.

Conclusion

The scAAV8-hOTCco vector we have developed may hold significant potential for treating neonatal-onset OTCD in humans.

Characterization of newborn OTC-KO pups (P1)

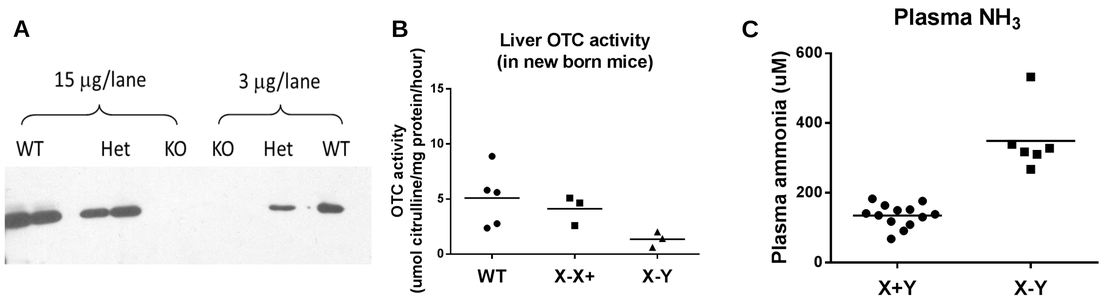


Figure 2. Characterization of newborn OTC-KO pups (P1).

A. Western analysis on liver lysate from P1 pups. No OTC protein is detected in the liver of OTC-KO pups.
B. OTC enzyme activity in P1 liver.
C. Plasma ammonia levels are elevated in P1 OTC-KO pups. ***p < 0.001.

Short-term rescue of OTC-KO pups by neonatal gene therapy

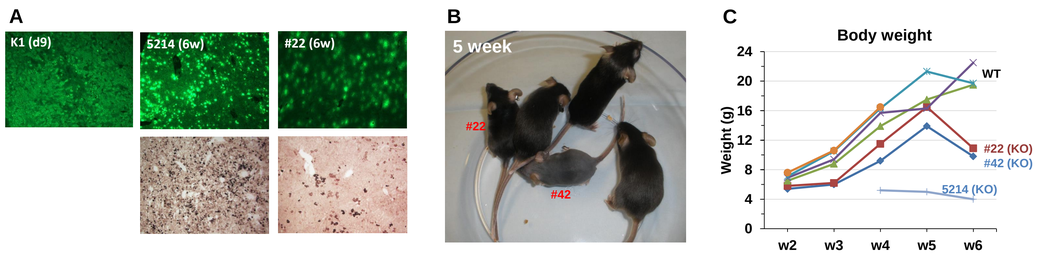


Figure 3. Successful rescue of OTC KO mice by neonatal gene therapy.

Male pups were retrieved from timed mating dams on the due date and immediately after revival, they received scAAV8-hOTC vector treatment via the temporal vein Mice were euthanized at various time points for analysis.

A. Expression of hOTC in livers harvested from rescued OTC KO mice shown by OTC immunostaining (top panel) and OTC enzyme activity staining (lower panel).
B. Five week old OTC KO mice (#22 and #42) and their WT littermates. Rescued OTC KO mice displayed a temporary phonotype of sparse-fur and abnormal skin (spf-ash).
C. Rescued OTC KO had lower body weight compared to the WT littermates.

Table 1. Summarized information on rescued OTC KO mice.

Animal ID (KO)	K1	5214	#22	#42	WT
Vector dose (GC/pup)	$1x10^{11}$	$3x10^{10}$	$3x10^{10}$	$3x10^{10}$	-
Age at euthanasia	d9	6w	6w	6w* (F.D.)	6w
Liver OTC activity (% of WT)	1147	39	14	n.a.*	~100
Urine orotic acid (umol/mmol of Cr)	n.a.	74 (4w)	45 (4w) 995 (5w)	67 (4w)	~20
Plasma NH3 (uM)	n.a.	n.a.	728 (6w)	n.a.	~50
Vector GC/cell	525	3.9	1.7	n.a.*	-

* #42 was found dead (F.D.) at 6 week

Long-term rescue of OTC KO mice

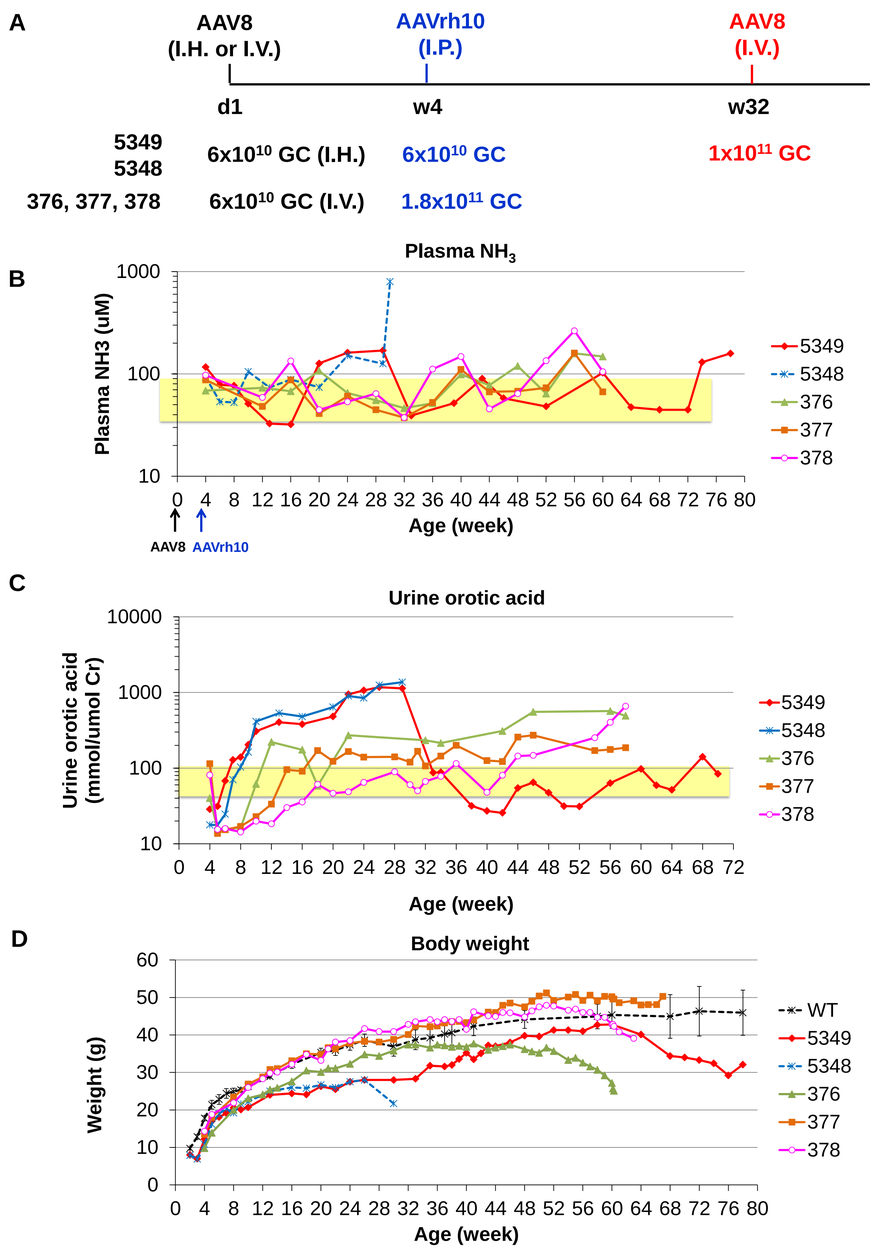
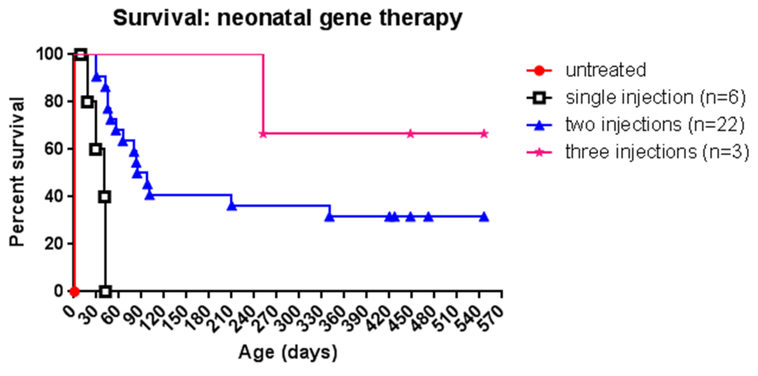


Figure 4. Long-term rescue of OTC KO mice by multiple treatments with AAV vectors.

Male pups were retrieved from timed mating dams on the due date and immediately after revival, they received scAAV8-hOTC vector treatment via the temporal vein or direct intrahepatic injection. Rescued OTC KO mice received a second vector injection (AAVrh10.hOTC) at the age of 4 weeks. One of the mice also received a third vector treatment at the age of 32 weeks. Time line and vector serotypes and doses are shown in (A). Rescued OTC KO mice were monitored by measurement of plasma NH_3 levels (B), urine orotic acid levels (C), and body weight (D).Normal ranges are indicated by yellow shade.

Survival curve



Future Direction

The Perelman School of Medicine at the University of Pennsylvania (PENN) is working with Dimension Therapeutics to bring gene therapy for OTC deficiency to the clinic.

Acknowledgements

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Disclosure

J.M. Wilson is an advisor to REGENX Biosciences and Dimension Therapeutics, and is a founder of, holds equity in, and receives grants from REGENX Biosciences and Dimension Therapeutics; in addition, he is a consultant to several biopharmaceutical companies and is an inventor on patents licensed to various biopharmaceutical companies.