Poster #5090

A CNS-targeted gene therapy for the treatment of severe pediatric obesity

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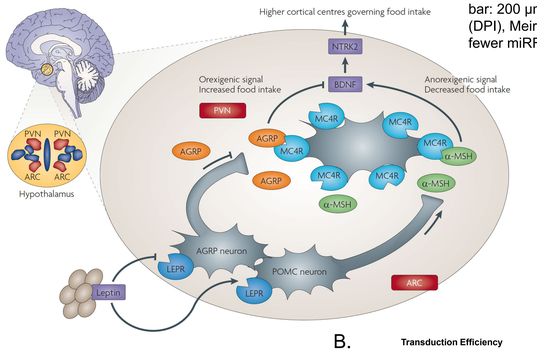
Developing an AAV-BDNF gene therapy for metabolic disorders

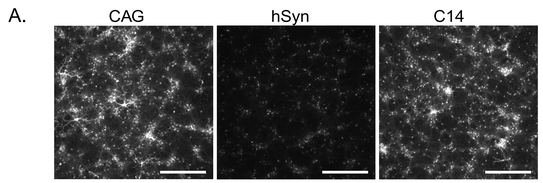
- **Elevated leptin signals** melanocortin 4 receptor (**MC4R)** expressing neurons to release brain-derived neurotrophic factor (**BDNF**).
- BDNF signals through its receptor **TrkB** and leads to decreased food intake.
- BDNF haploinsufficiency or mutations in MC4R cause **severe pediatric obesity** in humans.
- **Overexpression of BDNF** in various genetic and environmental obesity mouse models can **rescue** a wide range of **obesity-related phenotypes**.

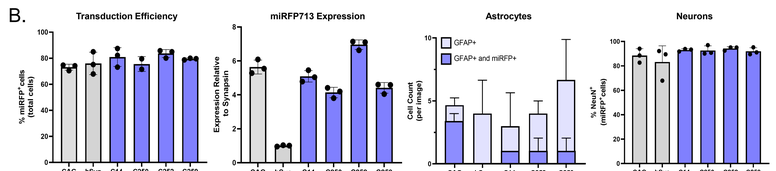


In the ventromedial hypothalamus, the leptin-proopiomelanocortin pathway initiates feeding or fasting through two opposing neuronal populations. In a fed state, elevated leptin signals a decrease in food intake via the release of BDNF from MC4R expressing neurons[1]. BDNF haploinsufficiency or de novo mutations in MC4R can cause severe obesity in human patients[2-4]. Compared to current therapeutic approaches such as glucagon-like peptide 1 (GLP1) agonists and MC4R agonists, using an adeno-associated virus (AAV)-based gene therapy to deliver BDNF provides more durable and significant effects on patients with MC4R deficiency, including patients with homozygous mutations. Here, we show an optimized gene therapy that induces a 19-fold increase in BDNF expression *in vivo* compared to a previously published construct[5] and over 143-fold higher expression than endogenous levels. With such a high expressing construct, we can reach drug efficacy at a lower viral vector dose.

Rationally designed MeiraGTx neuronal promoters show potent expression in primary cortical neurons

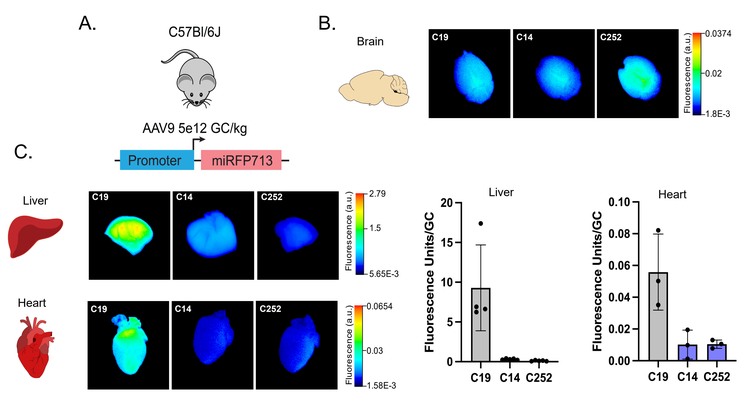
Expression and specificity of MeiraGTx promoters in primary neuronal culture. On day 4 *in vitro*, primary mouse cortical neurons were transduced at 50,000 MOI with AAV9-miRFP713 driven by candidate promoter. **(A)** After 7 days, neurons were imaged to assess miRFP713 expression. Scale bar: 200 µm. **(B).** Transduction efficiency is consistent across constructs. At 7 days post infection (DPI), MeiraGTx promoters leads to higher expression of miRFP713 than synapsin (hSyn) and shows fewer miRFP713-positive astrocytes than CAG.



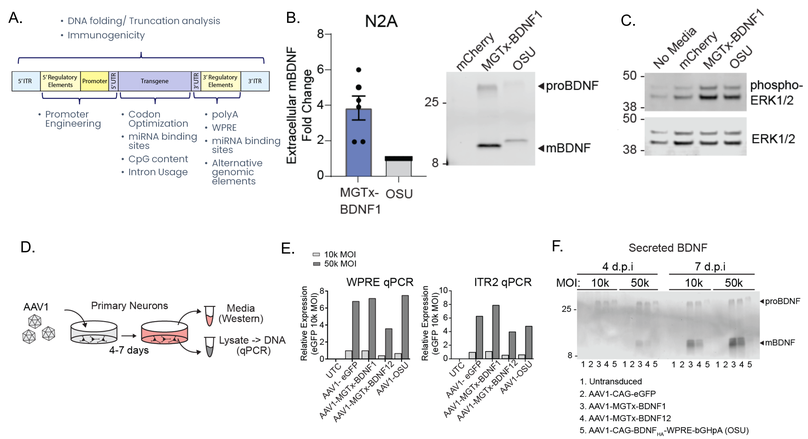


MeiraGTx promoters are specific to the CNS *in vivo*

Expression in peripheral mouse organs. (A) Male C57Bl/6J mice were injected intravenously with 5e12 GC/kg AAV9-miRFP713 driven by candidate promoters. After 4 weeks, fluorescence was measured for each organ *ex vivo* and genomes harvested for qPCR to quantify transduction. **(B**) Representative images of *ex vivo* fluorescence of whole brain showing higher expression driven by MeiraGTx promoters compared to CAG. **(C)** Peripheral tissue expression normalized to viral genomes shows low off-target expression in the mouse liver and heart driven by MeiraGTx promoters.

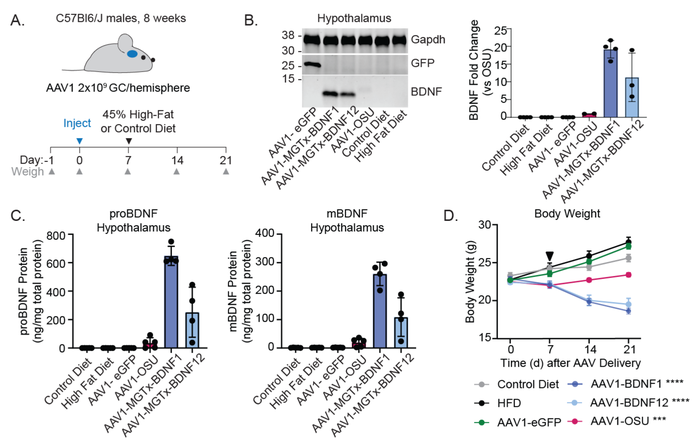


MeiraGTx vector design optimizations achieve superior expression *in vitro*



Vector design platform yields a high-expressing BDNF gene therapy. (A) Optimization pipeline for AAV-BDNF candidate design. **(B)** Six independent experiments show top candidate, MGTx-BDNF1, expresses 4-fold higher than an academic construct in N2A cells. **(C)** BDNF activity is assessed by treating primary cortical mouse neurons with conditioned media for 30 minutes and measuring the induction of phosphorylated ERK1/2. Codon optimization does not impair BDNF-dependent signaling in cultured neurons. **(D)** Primary cortical mouse neurons were transduced with AAV1 at 10,000 or 50,000 multiplicity of infection (MOI). Media was collected 4- and 7-days post infection (d.p.i) and prepared for western blot. DNA was isolated from neuronal lysates at 7 d.p.i. **(E)** Neuronal transduction was equivalent across groups as quantified by qPCR for viral genomes using probes for either the transgene (WPRE) and the ITR sequences. **(F)** Transduction with MGTx-BDNF1 and MGTx-BDNF12 *in vitro* leads to significantly higher BDNF expression in primary cortical neurons.

Expressing MGTx-BDNF in the VMH induces rapid weight loss in lean mice under HFD



Efficacy of MGTx-BDNF in a diet-induced obesity mouse model. (A) AAV1 (2E9 GC/hemisphere) was bilaterally injected into the hypothalamus of wild-type C57Bl6 male mice at 8 weeks of age and weighed weekly. After 7 days, animals were placed on 45% High-fat or matched control diet. After 21 days, the hypothalamus was dissected for protein analysis. **(B)** Western blot shows high expression of MGTx-BDNF constructs *in vivo*. **(C)** ELISA quantification of pro- and mBDNF in the hypothalamus confirms BDNF over-expression. **(D)** Weight change after AAV1 delivery in the diet-induced obesity mouse model. ***p <0.001; ****p<0.0001 One-Way ANOVA repeated measures

Conclusions

- MeiraGTx C14 promoter is as strong as the CAG promoter and has folds lower off-target expression in the mouse liver and heart.
- By combining the MeiraGTx C14 promoter and codon optimization, MGTx-BDNF1 expresses 4-fold higher than previously published construct in both primary mouse cortical neurons and N2A cell lines.
- BDNF-TrkB signaling through Erk1/2 MAPK phosphorylation indicates intact signaling function of MGTx-BDNF1.
- AAV1 delivery of BDNF1 or BDNF12 to ventromedial hypothalamus respectively leads to a 19-fold and 11-fold increase in expression compared to the previously published construct and around 143-fold increase compared to basal levels of BDNF *in vivo*.
- MGTx-BDNF1 and MGTx-BDNF12 cause steep and significant weight loss in a lean mouse model, indicating their enhanced potency.
- Continued weight loss on 45% HFD shows potential of MGTx-BDNF candidates on preventing weight regain in mouse model.
- By designing a highly-expressing BDNF gene therapy, we can drive efficacy at significantly lower viral vector doses.
- Together, these results identify a potent and effective gene therapy for rare pediatric obesity disorders such as MC4R deficiency.
- MeiraGTx intends to manufacture and make this construct available for physician use under our manufacturing Specials License in the UK.

References

1. Walley et al. Nat. Rev. Genetics 10, 431-442; 2009
2. Friedel et al. Am J Med Genet B Neuropsychiatr Genet 132B(1), 96-0; 2005
3. Han et al. N Engl J Med. 359(9), 918-927; 2008
4. Yeo et al. Nat Neurosci 7(11), 1187-9; 2004
5. Cao et al. Nat. Medicine, 15(4):447-54; 2009