Rescue of Amyloid Deposition Phenotype After Single-Treatment CRISPR/Cas9 Gene Editing in a Humanized Mouse Model of ATTR

Maria João Saraiva[1,2], Susete Costelha[1,2], Paula Gonçalves[1,2], Anabela Teixeira[1,2], Helena Sofia Martins[1,2], **Melissa Pink[3]**, Peter Bialek[3], Tanner Dirstine[3], Zachary Dymek[3], Jackson Eby[3], Noah Gardner[3], Bo Han[3], Ruchi Shah[3], Vaughn Walker[3], Reynald Lescarbeau[3], Jessica Seitzer[3], Cindy Shaw[3], Walter Strapps[3], Kristy Wood[3], Sean Burns[3], Yong Chang[3], Kevin Sloan[3]

[1]Instituto de Investigacao e Inovacao em Saude, Universidade do Porto, Porto, Portugal; [2]IBMC – Institute for Molecular and Cell Biology, Porto, Portugal; [3]Intellia Therapeutics, Cambridge, MA, USA

ABSTRACT

Transthyretin amyloidosis (ATTR) is a systemic, debilitating and fatal disease caused by accumulation of amyloid deposits of the transthyretin (TTR) protein in multiple tissues. The majority of TTR protein is produced by and secreted from the liver, and reduction of hepatic TTR protein is a clinically-validated approach to ATTR disease management. Several TTR-lowering drugs are in development, sharing a common mechanism of targeting *TTR* mRNA for destruction. These drugs will likely require chronic dosing over a patient's lifetime to maintain clinical benefit, due to the transient nature of the suppression. A more convenient approach for this patient population would be a permanent reduction of TTR expression, such as could potentially be provided by CRISPR/Cas9 gene editing with a single or limited number of treatments.

To explore the feasibility of sustained reduction of TTR expression in an ATTR disease model, we developed lipid nanoparticle (LNP) formulations containing a single chemically modified guide RNA specifically targeting the human *TTR* gene along with an mRNA encoding the *S. pyogenes* Cas9 nuclease. These formulations were evaluated in a well-established mouse model of hereditary ATTR amyloidosis that expresses the V30M pathogenic mutant form of human TTR protein and exhibits deposition of TTR within multiple tissues over time.

Our results demonstrate that a single administration of LNP-CRISPR/Cas9 plus led to durable and substantial reduction of *TTR* mRNA and protein expression in the liver, with a concomitant reduction in circulating serum TTR protein levels. Sustained reduction of hepatic TTR expression over a two-month period correlated with a marked reduction of TTR protein deposition in pathologically relevant tissues within the peripheral nervous system and GI tract. No changes in animal behavior or health were observed throughout the course of the study.

These findings highlight the potential of *in vivo* CRISPR/Cas9 gene editing and suggest that future therapies based on this platform may enable next-generation acute treatment paradigms for chronic diseases such as ATTR.

INTRODUCTION

ATTR (Transthyretin amyloidosis)

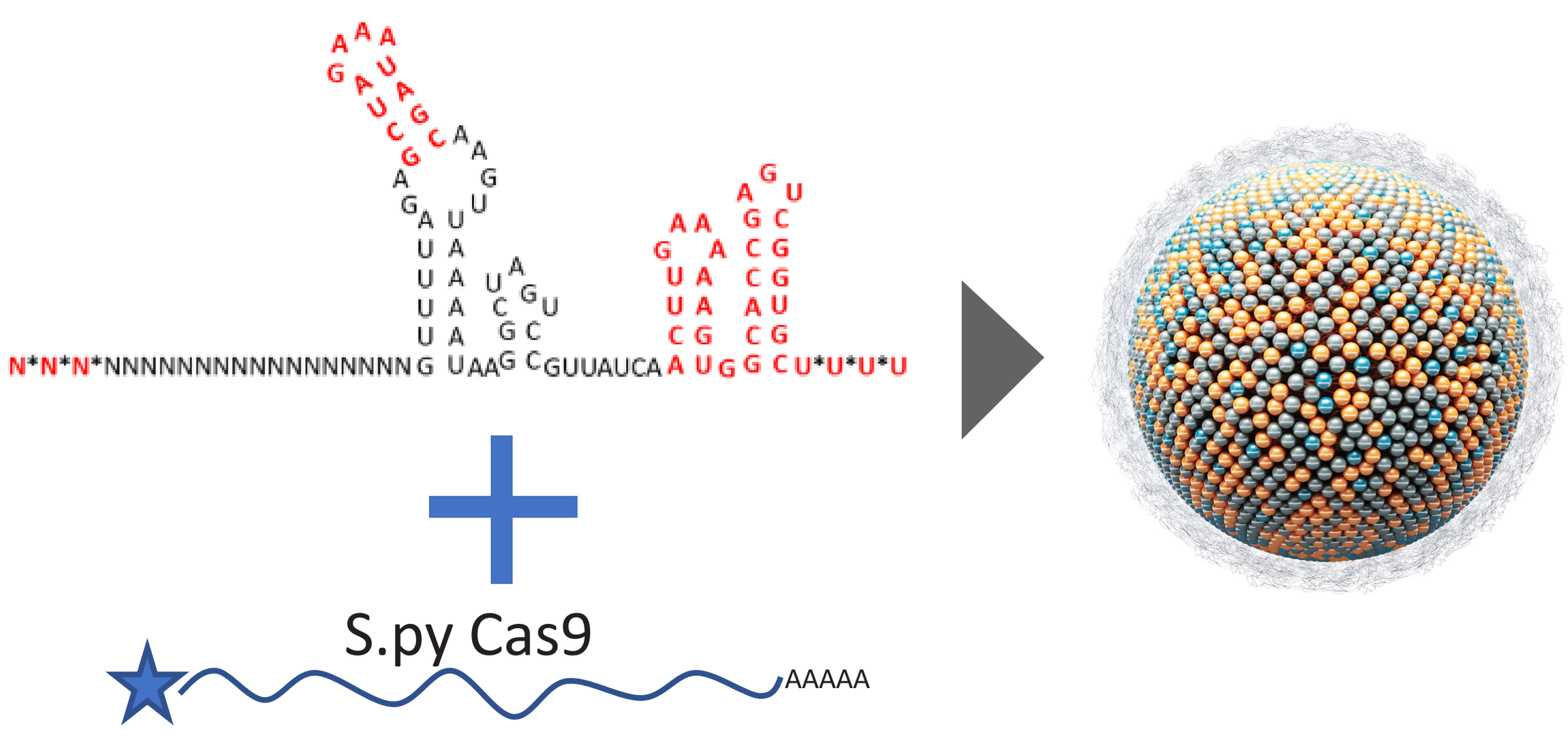
- Disease-causing mutations in the *TTR* gene can destabilize tetramer to monomer and drive aggregation and deposition of amyloid into a multitude of tissues. Amyloid deposits in the heart, nerves and other tissues can cause cardiomyopathy (h-ATTR-CM) and/or polyneuropathy (h-ATTR-PN). Deposition of amyloid may cause cytotoxicity, leading to organ damage.
- TTR is produced mainly by hepatocytes and normally circulates in the blood as a soluble homotetramer that facilitates transport of vitamin A, via retinol binding protein (RBP), as well as the thyroid hormone, thyroxine.
- TTR is also expressed centrally via the choroid plexus and can be detected in cerebrospinal fluid (CSF) albeit at much lower levels.
- Approximately ~100 mutations have been identified in the *TTR* gene which cause ATTR with the V30M mutation as one of the most common variants associated with the disease.
- HuTTR V30M mouse model recapitulates TTR deposition phenotype in tissues as well as in the nervous system as observed in patients with CM and PN.
- CRISPR/Cas9 gene editing may provide an alterative and permanent approach to disease treatment leading to improved symptomatology.
- We have previously demonstrated that a single administration of CRISPR/Cas9 lipid nanoparticles in wild type mice achieves robust and persistent reduction of mouse wild type TTR levels over 12 months (Finn et al., 2018).
- This study investigates the use of the CRISPR/Cas9 approach in a relevant disease mouse model of HuTTR V30M to potentially reverse amyloid depositions in tissues.

OBJECTIVES

- Evaluate the activity of a LNP formulated CRISPR/Cas9 to edit *TTR* locus in a phenotypic mouse model amyloidosis (HuTTR V30M).
- Correlate editing of *TTR* locus with TTR levels in serum and CSF to understand distribution of activity in relevant model.
- Quantify TTR amyloid deposition in tissues post editing.

METHODS

Test Article: Cas9 mRNA was *in vitro* transcribed and purified as previously described (Finn et al., 2018). Chemically synthesized sgRNA targeting human TTR and a non-targeting control were obtained from a commercial supplier. The sgRNA guide targets the human *TTR* gene within HuTTR V30M transgenic mouse model (see below). The chemically modified sgRNA and Cas9 mRNA were co-formulated with a proprietary lipid nanoparticle composition.



Mice: 4 – 5 month old transgenic mice, homozygous for the human mutant V30M TTR transgene in a mouse *TTR*-null background (Santos et al., 2003) were used. Transgenic mice contain approximately ~47 copies of HuTTR V30M. Animals were injected via tail vein with a single 1mg/kg dose of LNP formulated sgRNA/Cas9 mRNA with an n=10/group of either control or HuTTR sgRNA (LNP CRISPR/Cas9). At 8 weeks post treatment, the mice were euthanized for sample collection. TTR protein levels were measured in serum and CSF. A liver biopsy was obtained for genomic DNA editing levels. Other tissues (stomach, colon, sciatic nerve, dorsal root ganglion (DRG)) were collected and processed for immunohistochemistry as previously described (Gonçalves et al., 2014).

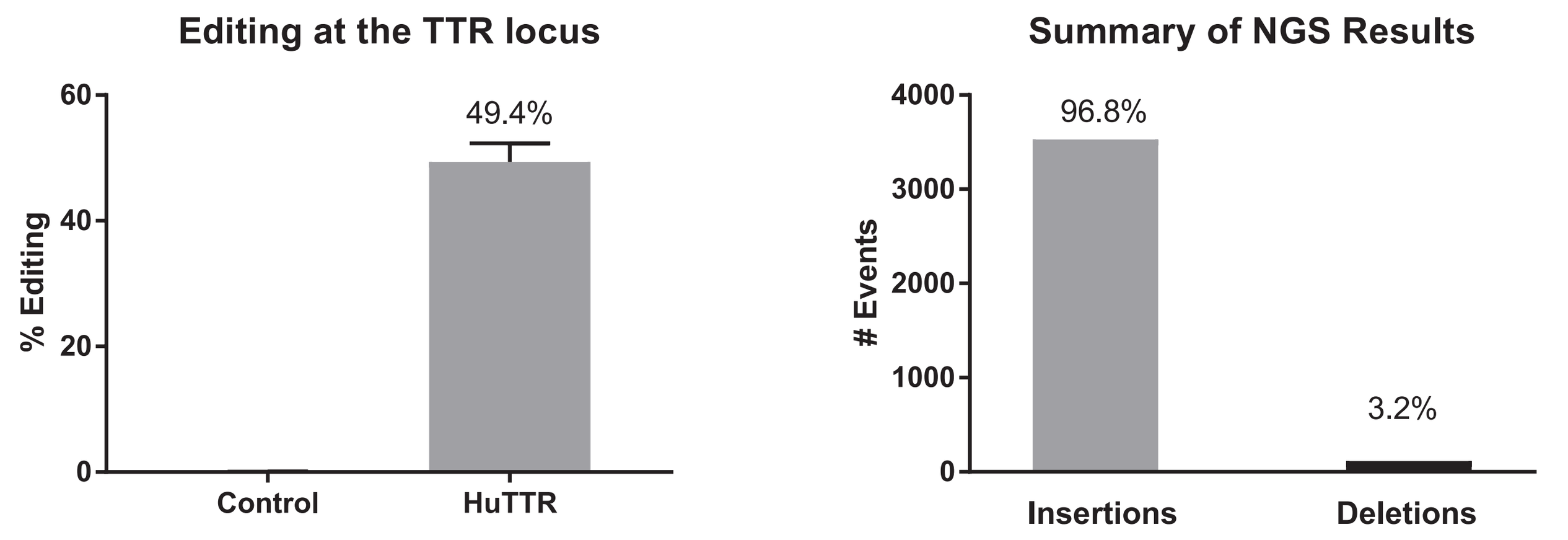
Liver editing by Next Generation Sequencing (NGS): Firstly, genomic DNA (gDNA) was isolated from livers by homogenizing a liver biopsy. Following isolation of gDNA, primers flanking the guide location were designed, amplification PCR performed, and 2x150 paired end sequencing is performed on an Illumina MiSeq. Reads were then stitched, and aligned to the human reference genome, and INDELs at the guide location were quantified as compared to wild-type reads to determine editing rate.

Circulating TTR levels in plasma and CSF by ELISA: The methods employed in the immunoassay to quantify human TTR in serum and CSF used a sandwich assay as previously described (Gonçalves et al., 2016).

Statistical analyses: Statistical comparison of data was performed using the Student t-test or One-way analysis of variance (ANOVA) followed by Bonferri´s post-test, with Graph Pad Prism software. Quantitative data are expressed as mean ± SEM. Statistical significance was established for p*<0.05, p**<0.01, p***<0.001.

RESULTS

Figure 1: Robust editing of TTR was observed in livers of HuTTR mice following a single dose of LNP CRISPR/Cas9

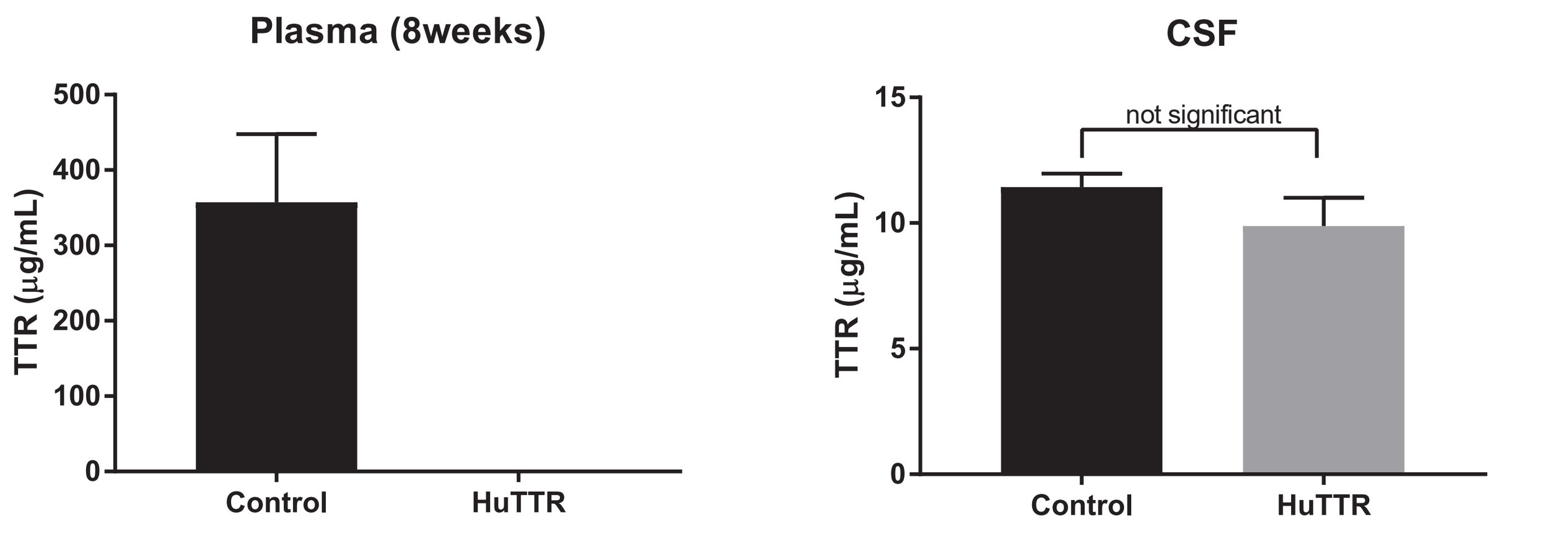


Genomic DNA was extracted from liver and editing of the HuTTR V30M locus was assessed by NGS. The editing percentage is defined as the total number of sequence reads with insertions or deletions over the total number of reads, including wild-type.

Key Finding:

- **HuTTR V30M mice treated with single administration of LNP CRISPR/Cas9 exhibited significant editing of the HuTTR V30M locus in the liver.**
- **Human sgRNA guide targets the human TTR.**
- **The % editing in liver homogenate likely underestimates the editing in hepatocytes since the uptake of LNPs is greater in hepatocytes, compared to other cell types in the liver.**

Figure 2: TTR protein levels were decreased in plasma but not in CSF from HuTTR V30M treated mice following a single dose

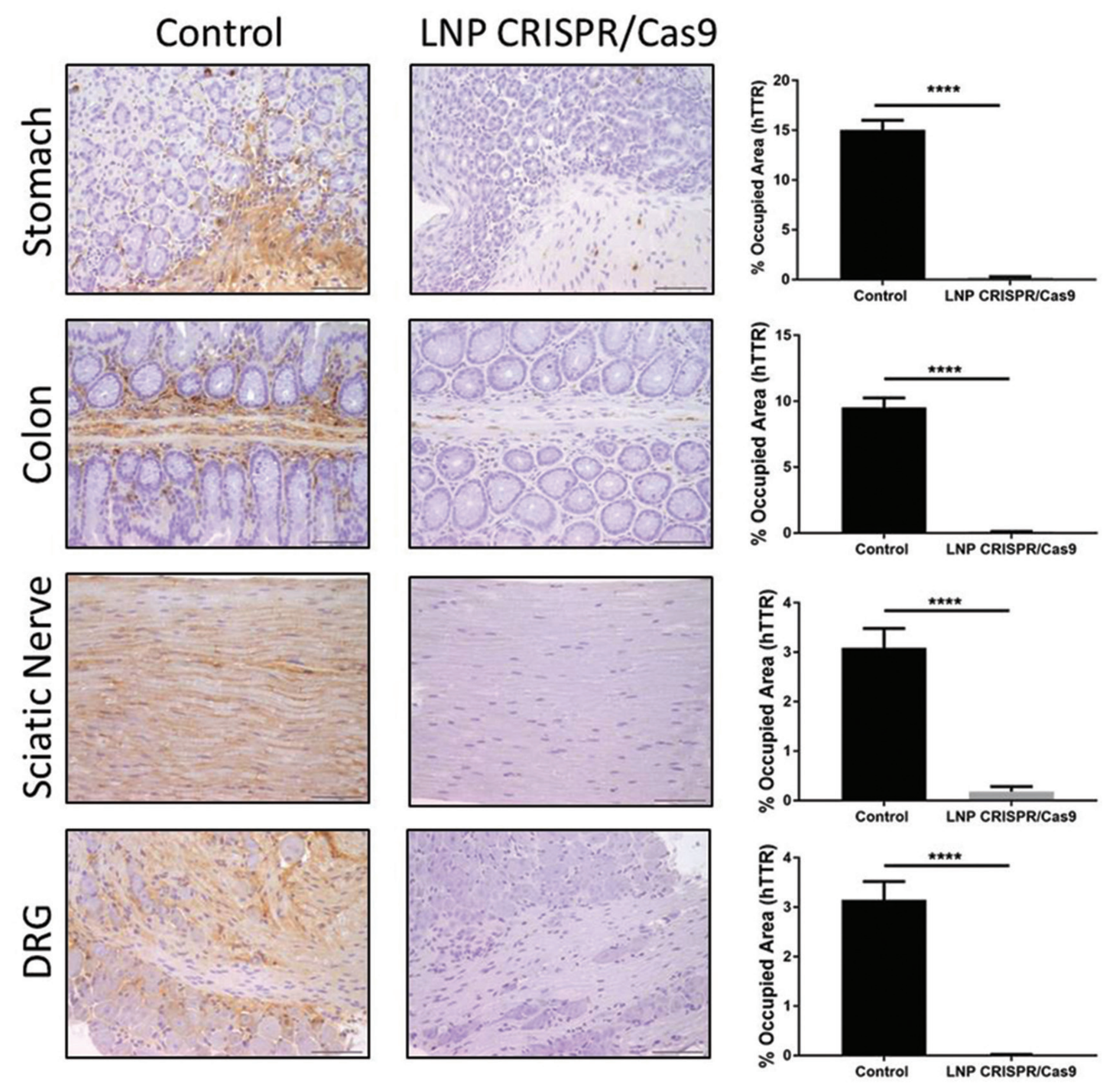


TTR concentrations in plasma and CSF were measured by ELISA 8 weeks post administration of LNP CRISPR/Cas9.

Key Finding:

- **A single administration of LNP formulated CRISPR/Cas9 resulted in >99% reduction of TTR in plasma at 8 weeks (p<0.001).**
- **Changes in peripheral TTR levels (plasma) had no effect on TTR levels in the CSF of mice treated with LNP CRISPR/Cas9.**

Figure 3: Amyloid deposition was significantly reduced in HuTTR V30M mice following a single dose of LNP CRISPR/Cas9



Semi-quantification of human TTR by immunohistochemistry in stomach, colon, sciatic nerve and DRGs.

Key Findings:

- **Control mice exhibited amyloid staining in tissues which resembles the pathophysiology observed in human subjects with ATTR.**
- **Decreasing serum TTR by editing the HuTTR V30M locus via LNP CRISPR/Cas9 results in dramatic decreased amyloid deposition in tissues. Approximately 85% or better reduction in TTR staining was observed across these tissues.**

CONCLUSIONS

- LNP formulated CRISPR/Cas9 resulted in robust *TTR* gene editing in liver, decreased serum TTR and dramatically less amyloid tissue deposition in HuTTR V30M mice which exhibit similar disease characteristics to hereditary ATTR.
- Decreased TTR levels in serum but not CSF confirmed that the activity of LNP CRISPR/Cas9 is restricted to reducing circulating levels of TTR and not affecting the TTR levels in the CNS.
- TTR deposition in tissues was decreased 8 weeks following a single administration of LNP CRISPR/Cas9. Decreased amyloid deposition may have the potential to halt and reverse the course of the polyneuropathy (h-ATTR-PM) and cardiomyopathy (h-ATTR-CM) in human subjects.
- Preclinical data suggests that reducing TTR levels via CRISPR/Cas9 may have the potential to ameliorating hereditary ATTR.