Systemic AAV Gene Therapy with Next Generation Engineered Capsids for Treatment of CNS and Cardiac Symptoms in Friedreich's Ataxia

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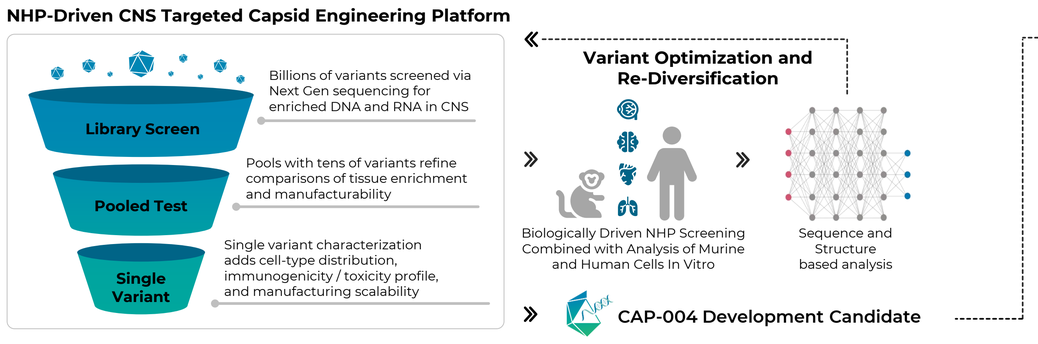
INTRODUCTION

FA is the most common genetic form of ataxia, which is most frequently caused by an intronic triplet repeat expansion in the *FXN* gene.[1-2] The repeat expansion diminishes the expression of the frataxin (FXN) protein causing progressive deficits in cellular respiration and cell death, with pronounced impact to functions controlled by non-regenerative cell types (neurons, cardiomyocytes).[3-4] There are currently few treatment options for this debilitating and life-threatening disease.[5]

Leveraging our high throughput directed evolution engineering platform, Capsida has identified a systemically administered (IV) capsid that achieves NHP brain-wide biodistribution transducing large percentages of neurons in key FA-related brain areas in addition to delivering therapeutically relevant levels of cardiac transduction. In an NHP study dosing a small pool of capsids simultaneously (N=3 NHP), the engineered capsid drives RNA expression levels ~100x higher than AAV9 in CNS, while maintaining similar RNA expression in cardiac tissue, and ~10x de-targeting in the liver. Importantly, Capsida has identified a novel blood-brain barrier receptor that binds to our engineered capsid. The receptor exhibits complete amino acid sequence homology between macaques and humans, thus de-risking clinical translation.

When administered IV as a single variant at a low to moderate dose (N=3 NHP), the engineered capsid delivering hFXN transduced more than 80% of cerebellar Purkinje cells, dentate nucleus neurons, motor neurons in the cortex and spinal cord, and nearly 30% of cardiac left ventricle tissue area, on average. Bulk protein levels in treated NHPs were 1.7x higher than endogenous levels in the left ventricle, and 8.2x higher than endogenous levels in the motor cortex by ELISA. Moreover, meaningful RNA expression levels were detected in the retina (~1E6 copies/ug RNA) by qPCR suggesting a potential benefit for sensory vision loss experienced by FA patients. De-targeting of the liver and other non-target tissues contributed to the favorable safety profile characterized by no adverse immunogenicity, clinical pathology, and histopathology findings. Together, these data demonstrate that a drug product driven by Capsida's engineered capsid produces therapeutically meaningful FXN expression in CNS, cardiac, and sensory regions impacted by disease and has the potential to become a best-in-class targeted therapy for the treatment of FA.

METHODS AND MATERIALS



NHPs: The CAP-004 development candidate was administered intravenously to WT cynomolgus macaques (N=3) at ~33 months of age. Analysis of CAP-004 transduction and expression was conducted after 4 weeks.

DNA/RNA: DNA biodistribution and mRNA expression were assessed using qPCR.

Protein: Exogenous FXN protein was detected in brain and cardiac tissue by western blot and quantified by ELISA.

Histology: The expression patterns of the HA-tagged FXN cargo was detected and quantified by anti-HA immunofluorescence.

RESULTS

IV administration of CAP-004 in NHPs achieves >100-fold higher expression in the brain and shows ~10-fold liver de-targeting relative to AAV9

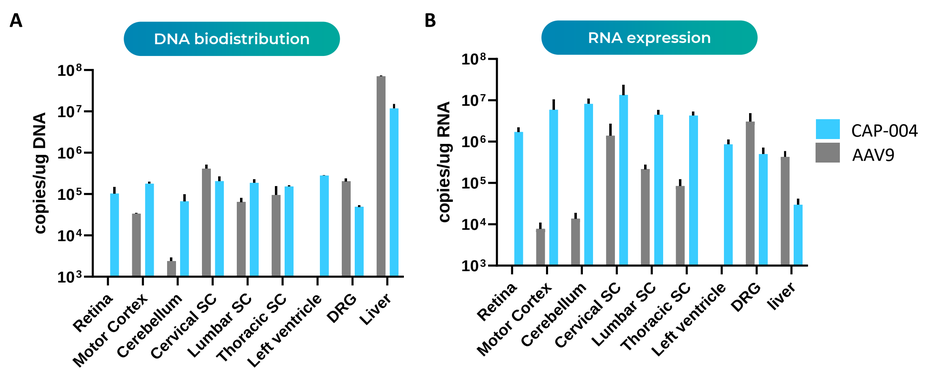


Figure 1. IV administration of CAP-004 in primates results in robust (A) DNA biodistribution and (B) mRNA expression across key areas of interest in the CNS and heart while de-targeting the liver to reduce safety risks associated with WT AAV9.

FXN protein in cerebral cortex and cardiac tissue from CAP-004 treated NHPs reveals robust exogenous FXN production and appropriate post-translational processing

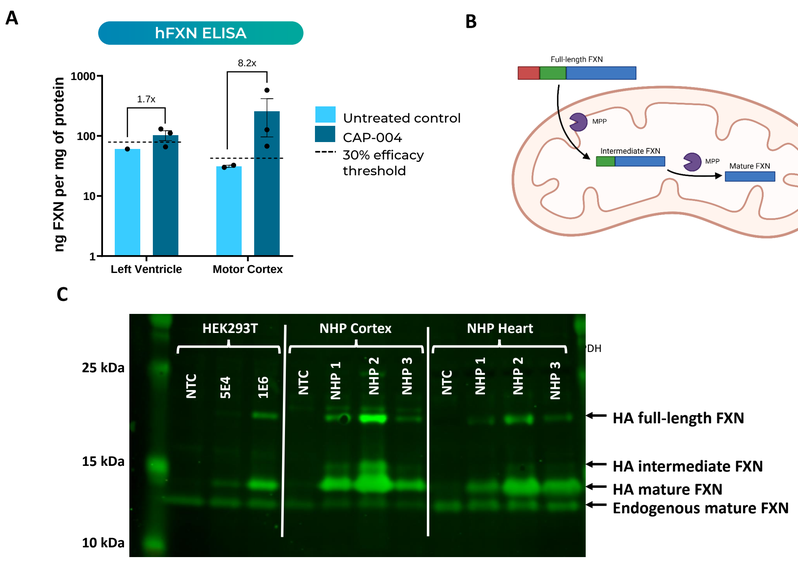


Figure 2. FXN protein quantification by (A) ELISA demonstrates that CAP-004 drives human FXN production beyond the therapeutic threshold of 30% of endogenous levels. (B) FXN is known to undergo two cleavage steps by mitochondrial processing peptidase (MPP) following mitochondrial import to produce mature FXN. (C) Western blot demonstrates the production of full-length, intermediate, and mature forms of FXN protein in CAP-004 treated human cells, NHP cerebral cortex and NHP heart tissue.

CAP-004 drives FXN-HA protein expression across central and peripheral tissues and cell types that are central to Friedreich's Ataxia symptoms and pathology

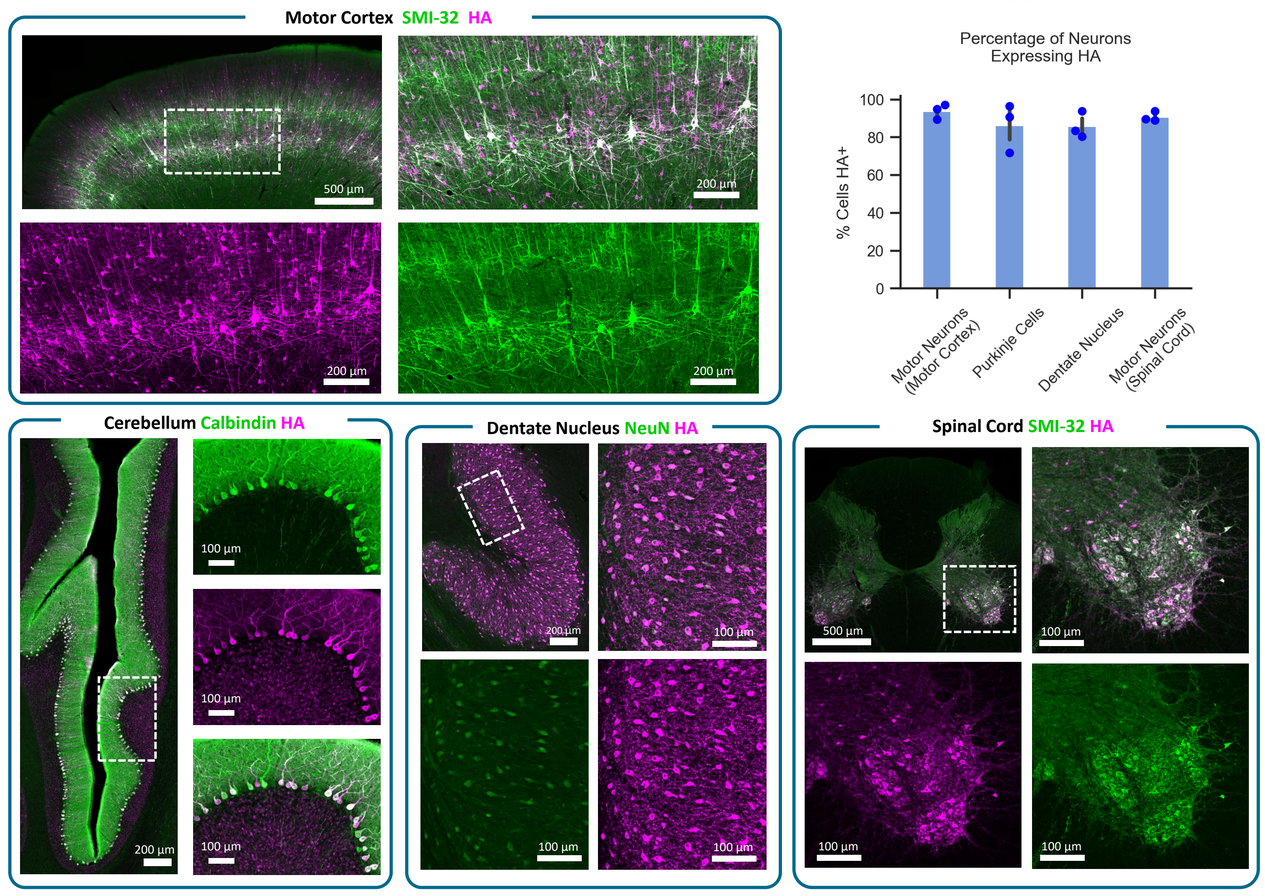


Figure 3. Neuronal cell type marker and anti-HA immunofluorescence demonstrates that CAP-004 transduces more than 80% (on average) upper motor neurons in the primary motor cortex, Purkinje cells in the cerebellum, neurons of the dentate nucleus of the cerebellum, and lower motor neurons of the spinal cord.

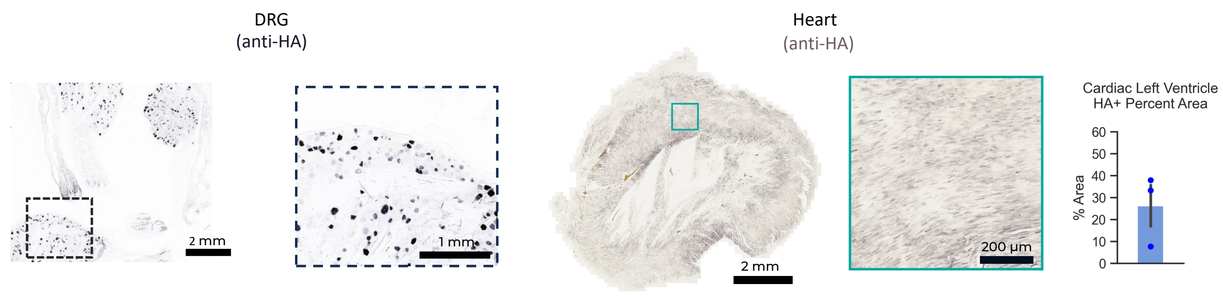


Figure 4. CAP-004 delivers FXN-HA expression to the dorsal root ganglia (DRG) and nearly 30% of heart left ventricle tissue of NHPs. Representative images of DRG (top) and heart (bottom) tissue from a CAP-004 treated NHP subjected to anti-HA immunohistochemistry.

CONCLUSION/DISCUSSION

- Capsida's engineered capsids bind to identified human BBB receptor and achieve superior dual transduction of CNS and periphery (DRG, heart, retina), while simultaneously de-targeting the liver compared to AAV9.
  - CAP-004 achieves therapeutically promising levels of human FXN protein expression in the CNS (greater than 80% of key neuron types and 8-fold endogenous levels of protein) and cardiac tissue (nearly 30% of left ventricle area and 1.7-fold endogenous levels of protein), thus making it a potentially best-in-class treatment for FA.
  - CAP-004 drives meaningful RNA expression in the retina suggesting a potential in treating sensory vision loss
  - CAP-004 is well tolerated and exhibits a favorable safety profile.
- Next steps:
  - We will perform further IND enabling work to support prospect for direct benefit to patients and explore additional dose ranges.

References

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