Efficient Neuronal Transduction of AAV2-derived CereAAV.YN Vector in Cynomolgus Macaque Brain without Liver Transduction by Systemic Injection.

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COI disclosure
Y. T, H. N, N. O, I, U and S. O are employees of TAKARA BIO INC., T. E is Operating Officer of TAKARA BIO INC.

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Abstract

In this study, we have evaluated the gene transduction capability of AAV2-derived mutant, CereAAV.YN vector in non-human primate model (Cynomolgus macaque). The CereAAV.YN vector carrying AcGFP gene as a reporter under the CAG promoter control was injected systemically into two male Cynomolgus macaques at a dose of 1e13 vg/kg. After 28 days, various tissues were collected and the gene transduction was evaluated by immunofluorescence assay. As a result, significant AcGFP expression was observed in the brain, spinal cord and heart, but not in the liver. Furthermore, immunostaining analysis showed that CereAAV.YN vector was highly transduced into neurons in the monkey brain, but not into astrocyte, oligodendrocyte, or microglia, similar to previous study in a rodent model. The neuronal transduction of the CereAAV.YN vector in the cerebral cortex occurred in more than 70-90% of neurons in the various region of the brain. Moreover, the AcGFP expression in the cerebral cortex was higher than in internal brain regions, such as the thalamus and putamen. On the other hand, systemic injection of CereAAV.YN vector did not show liver toxicity by determining ALT activity in the serum.
CereAAV.YN vector is a promising tool for both research and clinical studies targeting brain diseases without any liver failure. Currently, further studies are underway to reveal the mechanism of gene delivery across the blood brain barrier by CereAAV vectors.

Introduction

Fig. 1

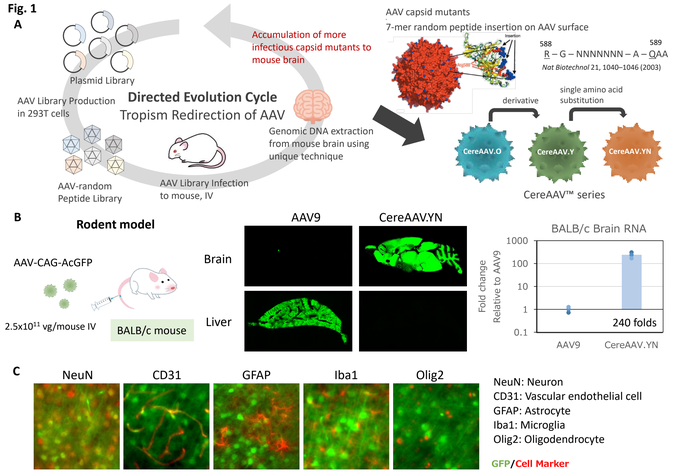

Figure 1. CereAAV.YN gene transduction in rodent model.
(A) Identification of CereAAV.YN vector by directed evolution for targeting mouse brain and amino-acid substitution in the capsid.
(B) AcGFP fluorescence by CereAAV.YN and AAV9 vectors transduction in mouse brain and liver 4 weeks after AAV injection. Quantification of AcGFP mRNA by CereAAV vectors transduction compared to AAV9 vector. The gene delivery capability of CereAAV.YN into mouse brain was about 240-folds higher than AAV9 in BALB/c mice by systemic injection.
(C) CereAAV.YN vector specifically delivers the gene into neurons in the mouse brain by intravenous administration.

Workflow of Cynomolgus Macaque Study of CereAAV.YN vector

Fig. 2

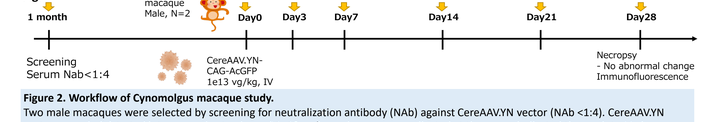

Figure 2. Workflow of Cynomolgus macaque study.
Two male macaques were selected by screening for neutralization antibody (NAb) against CereAAV.YN vector (NAb <1:4). CereAAV.YN vector was administrated intravenously at a dose of 1e13 vg/kg and serum was collected at the arrow-indicated time points. Various tissues were collected 28 days after CereAAV.YN injection.

Result 1: Vector Toxicity Evaluation

Fig. 3

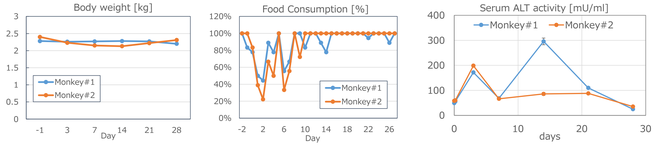

Figure 3. Evaluation of CereAAV.YN vector toxicity.
Evaluation of CereAAV.YN vector toxicity. Body weight, food consumption and ALT activity in serum were measured at the indicated time points.
The ALT activity was increased transiently correlated with decreased food consumption.

Result 2: CNS Gene Transduction

Fig. 4

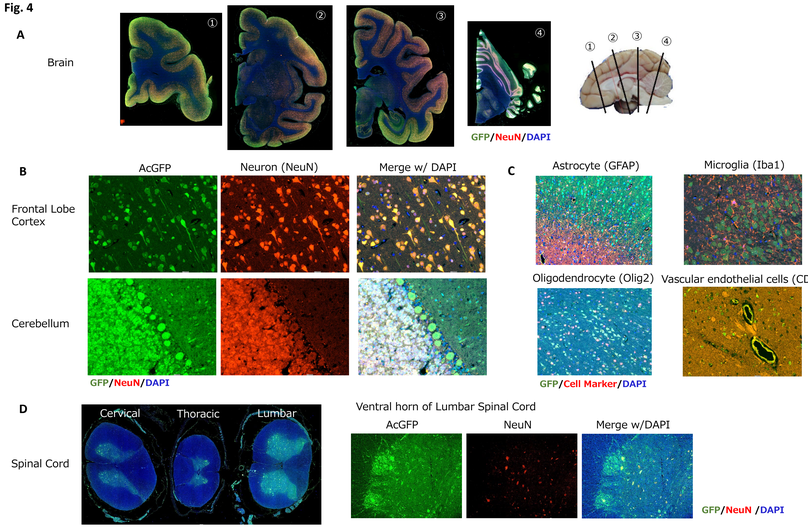

Figure 4. CereAAV.YN vector specific gene delivery to neurons in macaque brain and spinal cord.
Tissues were stained with antibodies to GFP and cellular markers for neurons (NeuN), astrocytes (GFAP), microglia (Iba1), oligodendrocytes (Olig-2) and vasculature endothelial cells (CD31). (A) The higher AcGFP expression was detected in external region of the brain, such as cortex than internal region such as putamen.
The AcGFP expression was highly merged with NeuN protein in neurons (B) and possibly merged with CD31 in vascular endothelial cells, but not GFAP in astrocytes, Iba1 in microglia and Olig2 in oligodendrocytes (C). (D) AcGFP expression was also merged with NueN cell marker in spinal cord.

Conclusions: CereAAV.YN vector Characterization

	Mouse	Macaque
Transduction target	Neuron / Vascular Endothelial cell	Neuron / Vascular Endothelial cell
Transduction efficiency	240-folds higher than AAV9	70-90% Neuron
Liver Toxicity	No abnormality	Low

Result 3: Quantification of Neuronal Gene Transduction

Fig. 5

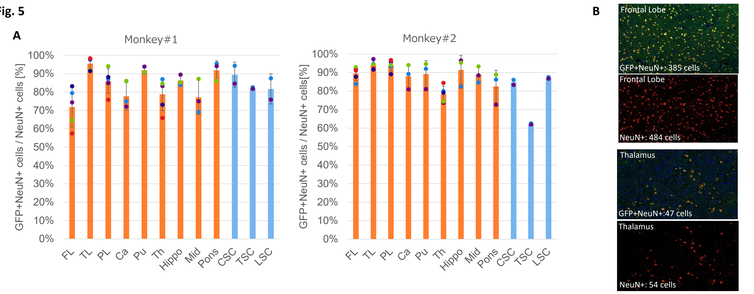

Figure 5. Quantification of the neuronal transduction efficiency of CereAAV.YN vectors in the macaque brain and spinal cord.
(A) The gene transduction efficiency in neurons was quantified by manually counting the numbers of GFP/NeuN double-positive cells and NeuN single-positive cells. The cell counting was performed in random 3-5 regions of the various areas of the brain and spinal cord. (FL: frontal lobe, TL: temporal lobe, PL: pariental lobe, Ca: caudate, Pu: Putamen, Th: thalamus, Hippo: hippocampus, Mid: midbrain, CSC: cervical spinal cord, TSC: thoracic spinal cord, LSC: lumbar spinal cord.)
(B) Examples of cell counting of GFP/NeuN double positive cells and NeuN single positive cells in frontal lobe and thalamus.
Almost 70-90% of neuron in the brain and spinal cord were transduced by CereAAV.YN vector systemic injection.

Result 4: Biodistribution

Fig. 6

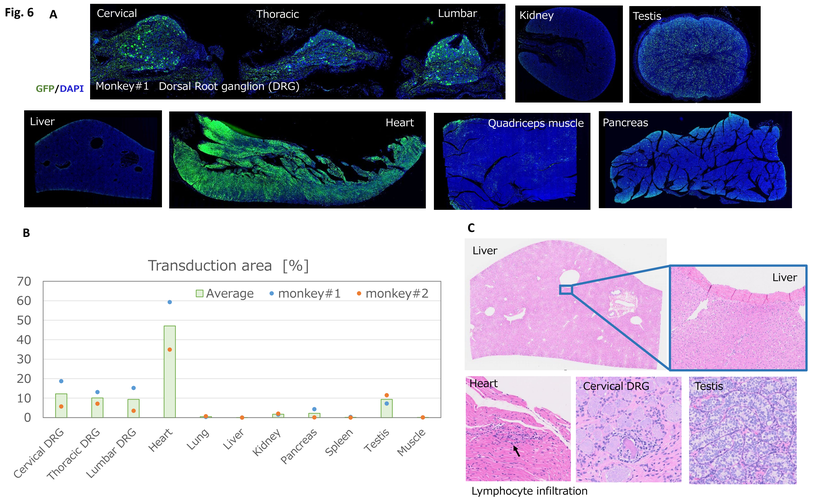

Figure 6. The gene transduction of CereAAV.YN vector in the macaque organs.
(A) Tissues were stained with antibody to GFP and DAPI for determining CereAAV.YN vector biodistribution. The AcGFP expression was observed in dorsal root ganglions, kidney, testis and heart, but not liver. (B) The transduction efficiency was determined by measuring GFP positive area relative to the total tissue area. About 35-60% of the heart and 10% of DRGs and testis were transduced by systemic injection of CereAAV.YN vector.
(C) hematoxylin and eosin (HE) staining images. Arrow shows lymphocyte infiltration in the heart. No abnormality was observed in the liver.
These result indicate that systemic injection of CereAAV.YN vector showed low hepatotoxicity in Cynomolgus macaque.