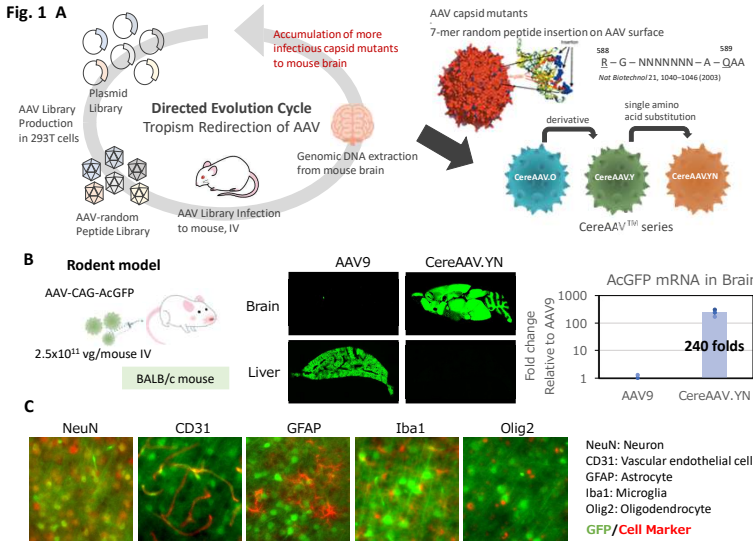


## Abstract

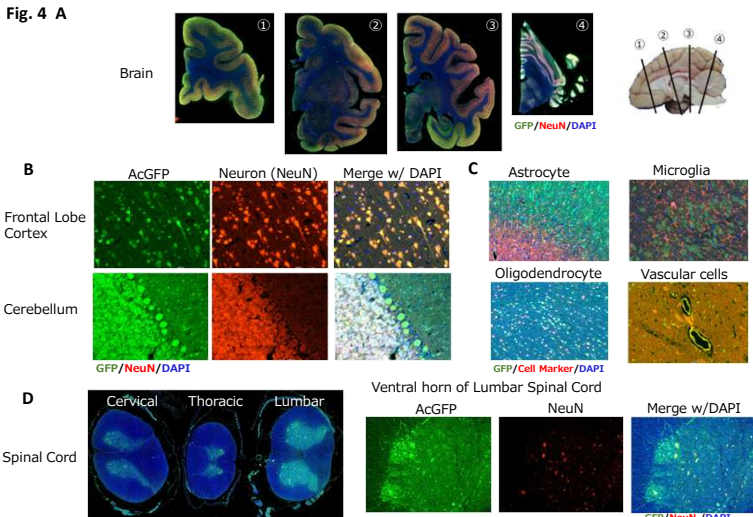
To date, we have developed an AAV2 capsid mutant, CereAAV.O, and demonstrated efficient gene delivery to the brain by systemic administration in mouse and non-human primate models. Furthermore, we have identified a novel CereAAV mutant (designated CereAAV.YN) that dramatically improves the efficiency of gene transfer to mouse brain compared to CereAAV.O and AAV9. In this study, to analyze the gene transduction capability of the CereAAV.YN vector in a non-human primate model (cynomolgus macaque), we systematically administered the CereAAV.YN vector carrying the AcGFP gene as a reporter under the control of the CAG promoter to two male cynomolgus macaques at a dose of 1e13 vg/kg. After 28 days, various tissues were collected, and gene transduction was evaluated by immunofluorescence assay. As a result, significant AcGFP expression was observed in the brain, spinal cord, and heart by immunostaining studies, but not in the liver. The AcGFP expression was observed in a broad region of the brain, but the AcGFP expression in the brain cortex was higher than in the internal brain regions such as the thalamus and putamen. Furthermore, the gene transduction of CereAAV.YN vector was observed predominantly in neurons, but not in astrocytes, oligodendrocytes, or microglia. Quantitative analysis of the gene transduction efficiency of CereAAV.YN vector into neurons revealed that 70%-90% of neurons in various regions of the brain were transduced. We also evaluated the toxicity of systemic administration of CereAAV.YN vector. Serum alanine aminotransferase (ALT) activity, a marker of liver toxicity, was measured continuously during the study period. The ALT activity increased temporarily on day 3 after administration but decreased to normal levels on day 7. In addition, in one macaque, ALT activity increased again temporarily on day 14. Ultimately, on day 28, both macaques showed normal levels of serum ALT activity, and no liver abnormalities were observed in the necropsy samples. These results indicate that the CereAAV.YN vector is a promising tool targeting brain diseases without causing liver damage. Further studies are currently underway to elucidate the mechanism of gene transfer across the blood-brain barrier by CereAAV.YN vectors.

### Introduction

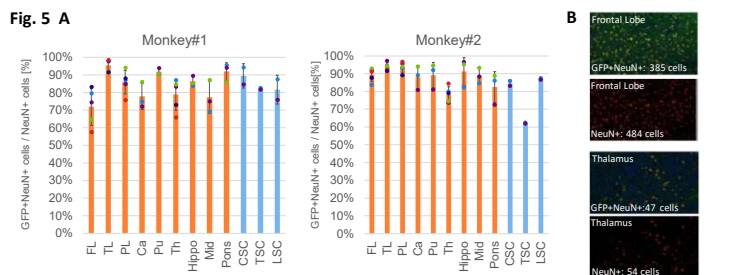


**Fig. 1. CereAAV.YN gene transduction in rodent model.** (A) Identification of CereAAV.YN vector by directed evolution for targeting mouse brain and amino-acid substitution in the capsid. (B) AcGFP fluorescence by CereAAV.YN and AAV9 vectors transduction in mouse brain and liver 4 weeks after AAV injection. Quantification of AcGFP mRNA by CereAAV.YN 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) Immunofluorescence assay using various cell markers in the mouse brain. **CereAAV.YN vector specifically delivers the gene into neurons in the mouse brain by intravenous administration.**

### Result 2: CNS Gene Transduction

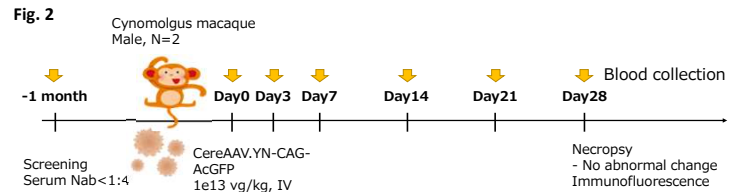


**Fig. 4. CereAAV.YN vector specific gene delivery to neurons in macaque brain and spinal cord.** Tissues were stained with antibodies to GFP and cellular markers for neurons (NeuN), astrocytes (GFAP), microglia (Iba1), oligodendrocytes (Olig-2) and vasculature endothelial cells (CD31). (A) The higher 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 NeuN cell marker in spinal cord. **CereAAV.YN vector targeted neurons in Cynomolgus macaque. CereAAV.YN vector delivers the gene into neurons and vascular endothelial cells in the monkey brain by intravenous administration.**



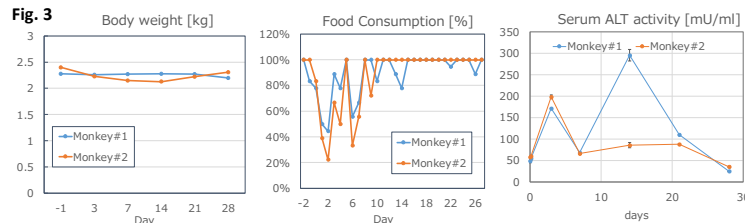
**Fig. 5. Quantification of the neuronal transduction efficiency of CereAAV.YN vectors in the macaque brain and spinal cord.** (A) The gene transduction efficiency in neurons was quantified by manually counting the numbers of GFP/NeuN double-positive cells and NeuN single-positive cells. The cell counting was performed in random 3-5 regions of the various areas of the brain and spinal cord. (FL: frontal lobe, TL: temporal lobe, PL: parietal 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.**

### Workflow of Cynomolgus Macaque Study of CereAAV.YN vector



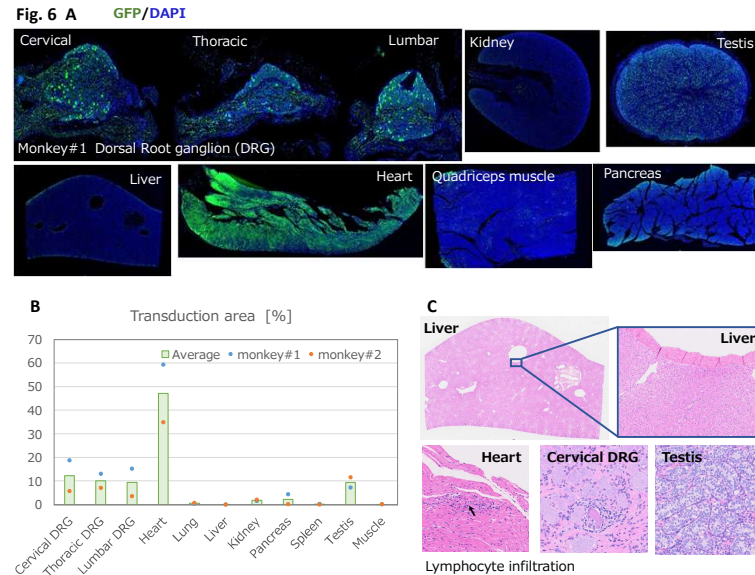
**Fig. 2. Workflow of Cynomolgus macaque study.** Two male macaques were selected by screening for neutralization antibody (NAb) against CereAAV.YN vector (Nab <1:4). CereAAV.YN vector was administered intravenously at a dose of 1e13 vg/kg and serum was collected at the arrow-indicated time points. Various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

### Result 1: Vector Toxicity Evaluation



**Fig. 3. Evaluation of CereAAV.YN vector toxicity.** Body weight, food consumption and alanine aminotransferase (ALT) activity in serum were measured at the indicated time points. **Transient elevation of ALT activity and decreased food consumption were observed in response to systemic administration of CereAAV.YN vector in Cynomolgus macaques.**

### Result 3: Biodistribution



**Fig. 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. **The systemic administration of CereAAV.YN vector demonstrated liver detargeting function in Cynomolgus macaques.**

### 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