Characterization of Brain Targeting CereAAV™ Gene Transduction In Cynomolgus Macaque And Further Improvement Of Gene Delivery Capability By Amino Acid Substitutions.

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

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Abstract

Recombinant adeno-associated virus vectors (AAVs) are widely used for gene delivery in research and therapy. AAV9 variants, such as AAV9-PHP.eB, are frequently employed for gene delivery into the central nervous system (CNS), while there are limited reports of efficient transduction of the CNS by AAV2 variants. To overcome the limitations of AAV2, we addressed to develop a novel brain-targeting AAV vectors based on AAV2 serotype. To date, we have demonstrated that CereAAV.O, which was obtained by directed evolution using random peptide-inserted AAV2 library, can efficiently transduce in mouse, and marmoset brain by systemic injection. Furthermore, we have identified a novel CereAAV.Y mutant with a specific and higher transduction efficiency in the mouse brain compared to CereAAV.O through a single amino acid substitution. Recently, Kawabata et al. have demonstrated that a single amino acid substitution, changing glutamine to asparagine at position 587 (Q587N) in the AAV-BR1 capsid, could increase the BBB permeability and redirect gene delivery form vesicular endothelial cells to neurons in the mouse brain.

In this study, we evaluated CereAAV.O gene transduction efficiency in cynomolgus macaque. We used barcoded AAV library technology to compare the AAV capsids in single monkey. As a result, CereAAV.O showed significant higher gene transduction efficiency in the monkey CNS compared to wild type AAV2 and AAV9 by intravenous administration.

Moreover, we attempted to improve the gene transduction of CereAAV.Y vector by introducing Q587N amino acid substitution (designated as CereAAV.YN). The gene delivery capability of CereAAV.YN into mouse brain was about 240-folds higher than AAV9 by intravenous administration. Furthermore, immunostaining analysis revealed that CereAAV.YN was mainly transduced into neurons in the cerebral cortex, but not astrocyte, oligodendrocyte and microglia. These results indicated that CereAAV.YN vectors may have a benefit to use as a transduction tool for research use, but also clinical studies against brain diseases.

Introduction

Fig. 1

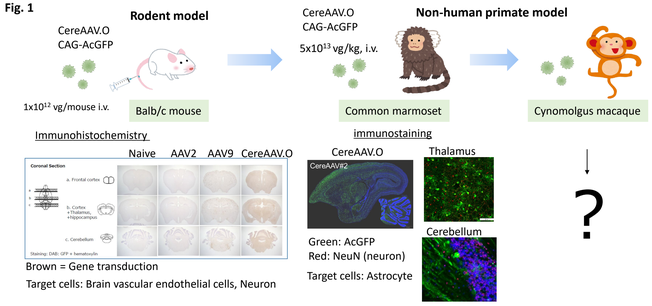

Figure 1. CereAAV.O gene transduction in rodent and non-human primate models.
CereAAV.O can efficiently transduce in mouse and marmoset brain by systemic injection. The gene delivery target by CereAAV.O systemic injection were neuronal cells and vascular endothelial cells in the mouse brain and astrocyte like cells in the marmoset brain.

Conclusions

- ✓ CereAAV.O showed more than 5-folds higher gene transduction efficiency to the CNS in cynomolgus macaque than AAV9.
- ✓ Amino acid substitutions into CereAAV.O, named as CereAAV.YN, improved the gene transduction efficiency and showed 240-folds higher and specific gene transduction in mouse brain compared to AAV9.
- ✓ CereAAV.YN can mainly transduce into neuron in the mouse brain.

Result.1: Evaluation of CereAAV.O biodistribution in Cynomolgus macaque using barcoded AAV library

Fig. 2

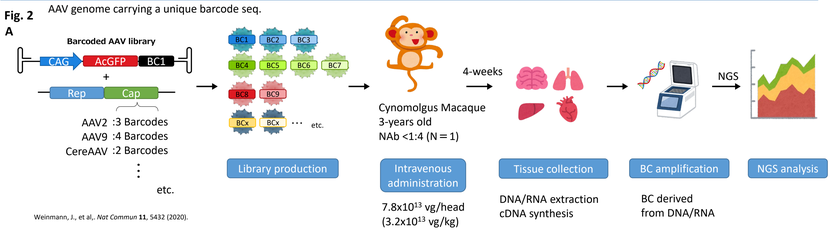

Figure 2. Characterization of CereAAV.O gene delivery in cynomolgus macaque. (A) Workflow of barcoded AAV library experiment in cynomolgus macaque. (B) Enrichment score by NGS analysis indicating the transcription efficiency of AAV2 and CereAAV.O compared to AAV9. The barcode frequencies were calculated from the read counts of the barcodes in the collected tissue RNA and in the input AAV library DNA. The frequency of AAV2 and CereAAV against the AAV9 was normalized as the enrichment score. The transcriptional efficiency of CereAAV.O was about 5-folds higher than that of AAV9 in the brain
This result indicated that gene transduction efficiency of CereAAV.O was higher than AAV9 in cynomolgus macaque brain.

Result.2:Improvement the transduction efficiency by amino acid substitutions in CereAAV.O

Fig. 3

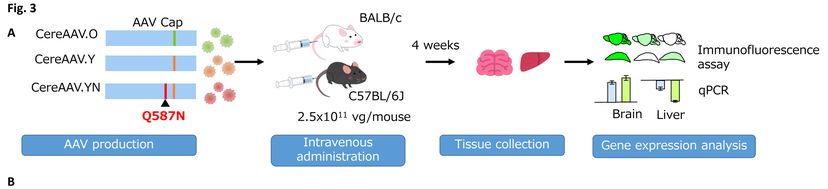

Figure 3. Experimental design for evaluation of CereAAV transduction in rodent model.
(A) Workflow of mouse study. AAV vectors, packaging a AcGFP under the control of the CAG promoter, were intravenously injected into BALB/c and C57BL/6J mice at 2.5 x10¹¹ viral genomes per mouse. After 4 weeks, we collected brain and liver tissues and then performed immunofluorescence staining assay and gene expression analysis by qPCR.
(B) Comparison of AAV production between wild type AAV2 and CereAAV variants (CereAAV.O, CereAAV.Y and CereAAV.YN). Data are presented as mean ± SD (n = 9) ; *: $p < 0.01$ (Student's t test).
This result indicated that the virus production of CereAAV.YN was about 2-folds higher than that of AAV2.

Fig. 4

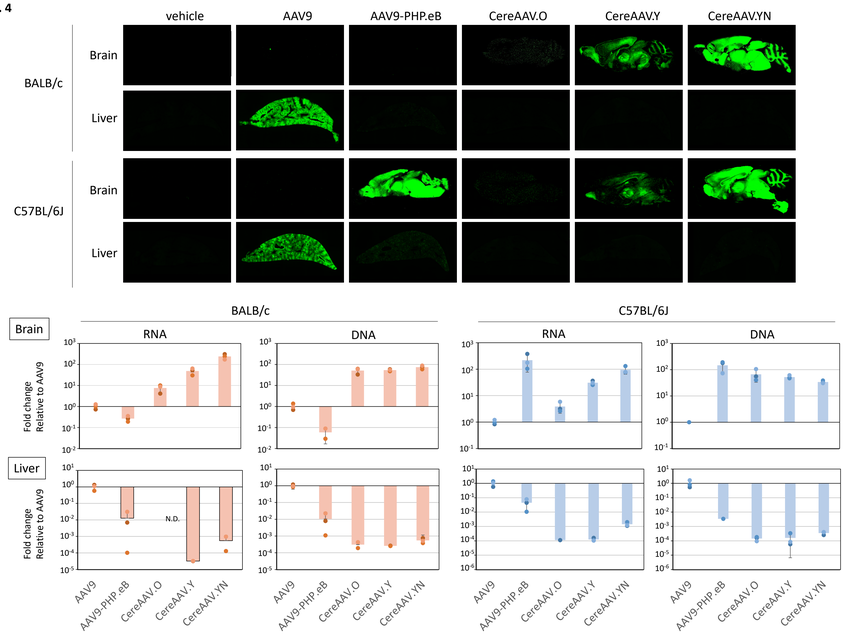

Figure 4. The transduction efficiency of CereAAV vectors in the mouse brain and liver.
(A) AcGFP fluorescence by AAV vectors transduction in mouse brain and liver of both BALB/c and C57BL/6j mice 4 weeks after AAV injection.
(B) Quantification of AcGFP mRNA and viral genomes by CereAAV vectors transduction compared to AAV9 vector. Data are presented as mean ± SD (n = 3).
The gene delivery capability of CereAAV.YN into mouse brain was about 240-folds higher than AAV9 in BALB/c mice by systemic injection.
The biodistribution of CereAAV.YN showed liver-detragreting phenotype compared to AAV9.

Fig. 5

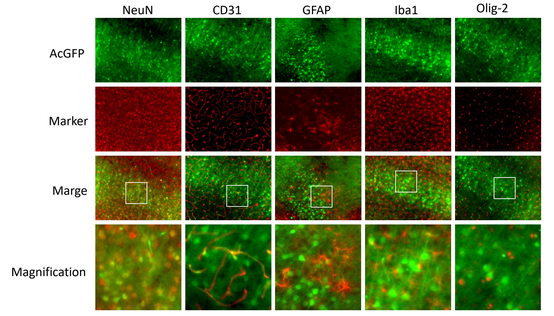

Figure 5. CereAAV.YN specific gene delivery to neurons in mouse brain.
Tissues were stained with antibodies to cellular markers for neurons (NeuN), microvasculature endothelial cells (CD31), astrocytes (GFAP), microglia (Iba1), oligodendrocytes (Olig-2). Significant co-localization of AcGFP fluorescence and NeuN cell marker was observed in the brain cortex, indicating that CereAAV.YN specifically delivers the gene into neurons in the mouse brain by intravenous administration.