# Title:

CRISPR/Cas9-Based Development of *progressive hydrocephaly (prh)* Rat Model of Hydrocephalus

# Authors:

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# Abstract:

INTRODUCTION: We recently discovered a gene mutation within *Coiled-coil domain containing protein 39 (Ccdc39)* gene that is responsible for development of congenital hydrocephalus in the *progressive hydrocephaly* (*prh*) mutant mouse. To address the use of surgical and imaging technology in larger animal models, we used CRISPR/Cas9 genome editing system to introduce the *prh* mutation into rats.

METHODS: Guide RNA oligomers and donor oligo DNA that create the *prh* mutation and components of CRISPR/Cas9 system were injected into fertilized Sprague Dawley rat embryos. The transplanted embryos in recipient pseudo-pregnant female rats were genotyped for evidence of gene manipulation in *Ccdc39* gene in Sanger sequencing after birth and their brain histology was analyzed.

RESULTS: Of the 18 first filial (F1) rats born from CRISPR-modified embryos, two showed growth retardation, hydrocephalus with the dome-shaped head, and compound heterozygous mutation of the *Ccdc39* gene. A mosaic mutant out of nine rats was bred with wild-type rat to transmit only one mutation type to each offspring (F2). The heterozygous splice site mutation was confirmed in at least three F2 rats.

CONCLUSIONS: The severe hydrocephalus phenotype found in two F1 rats proved that the recessive mutation in *Ccdc39* is a causative mutation for the *prh* phenotype commonly in rodents. Establishment of *prh* rat colony was achieved by obtaining *Ccdc39* heterozygous rats. The homozygous *prh* rats will be obtained and used *in vivo* non-invasive imaging, such as diffusion tensor imaging before and after shunt surgery procedure, to model human congenital hydrocephalus cases.