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Scientists use CRISPR for first time to correct clotting in newborn and adult mice

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CRISPR/Cas9, a powerful genome editing tool, is showing promise for efficient correction of disease-causing mutations. For the first time,

researchers from the Perelman School of Medicine at the University of Pennsylvania have developed a dual gene therapy approach to deliver key components of a CRISPR/Cas9-mediated gene targeting system to mice to treat hemophilia B. This disorder is also called factor IX deficiency and is caused by a missing or defective clotting protein. Their research will be presented during the 58th Annual American Society of Hematology Meeting and Exposition in San Diego from December 3-6 (Abstract #1174).

Most single-gene diseases, such as hemophilia, are caused by different mutations scattered in a specific gene rather than a single predominant mutation, so the team needed to develop a vector that would be applicable for patients with any mutations. The study is a preclinical proof of concept using a universal CRISPR/Cas9 gene targeting approach that could be applied to majority of the patients with a specific disease, in this case hemophilia B. According to the Centers for Disease Control and Prevention, hemophilia in general occurs in approximately 1 in 5,000 live births and there are about 20,000 people with hemophilia in the United States.

"Basically, we cured the mice," said first author Lili Wang, PhD, a research associate professor in the Penn Gene Therapy Program (GTP). James M Wilson, MD, PhD, a professor of medicine and GTP director, is senior author on the study.

To validate this new approach, the team performed the experiment in a mouse model in which the clotting factor IX was knocked out. They used a two-vector approach, with vector 1 expressing the SaCas9 gene driven by a liver-specific promoter so that the gene-editing machinery homes to the liver, the natural site that produces clotting factor IX. Vector 2 is what makes this study different from previous CRISPR-based-gene-therapy studies in the Penn Gene Therapy Program. Vector 2 contains an RNA sequence that specifically targets a region at the

5-prime end of exon 2 of the mouse factor IX gene and a partial human factor IX cDNA sequence, which gives this approach more potency and accuracy.

The team used adeno-associated viral vectors to deliver these components to the mouse liver cells. The strategy they developed is based on CRISPR-mediated homologous recombination to insert the human cDNA into the factor IX location on the mouse genome.

"The targeted insertion leads to the expression of a chimeric hyperactive factor IX protein under the control of the native mouse factor IX promoter," Wang said.

Injection of the two vectors with increasing doses in newborn and adult knockout mice showed stable Factor IX activity at or above the normal levels over four months. Eight weeks after the vector treatment, a subgroup of the newborn and adult treated knockout mice were given a partial liver removal and all of them survived the procedure without any complications or interventions and continued to express factor IX at similar levels.

"This study provides convincing evidence for efficacy in a hemophilia B mouse model following in vivo genome editing by CRISPR/Cas9," Wang said. Yang Yang PhD, a visiting scientist in the Wilson lab, and John White, McMenamin Deirdre, and Peter Bell, PhD, all from Penn, are also coauthors.

Provided by Perelman School of Medicine at the University of Pennsylvania