Efficient and precise generation of Tay–Sachs disease model in rabbit by prime editing system

Yuqiang Qian¹, Ding Zhao¹, Tingting Sui¹, Mao Chen¹, Zhiquan Liu¹, Hongmei Liu¹, Tao Zhang¹, Siyu Chen¹, Liangxue Lai¹,² and Zhanjun Li³

Dear Editor,

Tay–Sachs disease (TSD) is a progressive neurodegenerative disorder due to an autosomal recessively inherited deficiency of β-hexosaminidase A (HexA). The four-bases (TATC) insertion in exon 11 of the HEXA (HEXA ins TATC) accounts for 80% of Tay–Sachs disease from the Ashkenazi Jewish population. However, no typical clinical phenotypes, such as neurological abnormalities, the restricted pattern of distribution of GM2-ganglioside and membranous cytoplasmic bodies in the brain, were observed in HEXA−/− mouse models, due to the difference in the ganglioside degradation pathways in mice and human. Thus, it is desired to generate an ideal animal model to accurately mimic HEXA ins TATC in TSD patients. CRISPR–Cas9 system-mediated HDR has been used to generate the mutation of HEXA ins TATC, however, low efficiency and high indels impede its application.

Recently Anzalone et al. described a “search-and-replace” genome editing technology named prime editing (PE) that mediates 12 possible base-to-base conversions, without requiring DSBs or donor DNA templates in human cells. In addition, a previous study showed that, compared to mice, the late onset of TSD in adult rabbits shared more similarities with human regarding physiology, anatomy, and genetics. Thus, we generated a novel TSD rabbit model using the PE system, and characterized the typical phenotype of muscle weakness, ataxia, and mental disorders in the HEXA ins TATC rabbit model.

We first validated the editing efficiencies of PEs (PE2, PE3, PE3b) in HEK293FT cells at fifteen loci: five loci for base insertion, eight loci for base substitutions, and two loci for base deletion (Supplementary Table S1). Sanger sequencing results showed that the base insertion at a frequency from 4% to 22% (Fig. 1a and Supplementary Fig. S2), the base substitutions at a frequency from 4% to 36%, and the base deletion at a frequency from 7% to 12% were determined using PEs (Supplementary Figs. S1 and S2), respectively. These results indicate that PEs were effective in generating base insertion, substitution, and deletion in HEK293FT cells.

Next, we tested the efficiency of the PE system in rabbit embryos at three gene loci of HEXA, HBB, and TYR, which are associated with clinical diseases in ClinVar data (Supplementary Table S2). Sanger sequencing results showed that 9 of 20 desired HEXA ins TATC were determined using PE2 with the efficiency of 4.1%–15.4%, while the efficiency is 8%–37.5% using PE3. In addition, 1 of 14 desired HBB with an efficiency of 10% and 1 of 10 desired TYR with an efficiency of 14% were generated using PE3, while there is no desired mutation was detected for these two sites using PE2 (Fig. 1b and Supplementary Fig. S3).

We then targeted the HEXA ins TATC to test the efficiency of the PegRNA PBS length (8–16 nt) and RT template length (10–18 nt) in rabbit embryos. TIDE analyzing revealed significantly higher editing efficiencies by using PegRNA with 12 nt PBS and 14 nt RT template (Fig. 1c, d and Supplementary Table S3). Additionally, the significantly increased undesired indels were determined by using CRISPR–Cas9 system-mediated HDR (Fig. 1e and Supplementary Tables S3, S8), which is consistent with the previous study. Thus, PE3 with 12 nt PBS and...
Fig. 1 (See legend on next page.)
14 nt RT template was used for the generation of HEXA ins TATC rabbits in the following study. The HEXA ins TATC introduces a premature termination codon (PTC) in exon 11, which leads to deficient activity of the hexosaminidase A (HexA) binding site. In this study, 2 of 4 HEXA ins TATC rabbits were determined using Sanger sequencing and targeted deep sequencing, with the 68.17% and 14.23% mutation efficiency for #1 and #2 pups, respectively (Fig. 1g). Furthermore, no sgRNA sequence-dependent off-target mutations in HEXA ins TATC rabbits were found by deep sequencing (Supplementary Fig. S4a, b), suggesting the accuracy of PE system-mediated HEXA ins TATC mutations in rabbits. Furthermore, the heritability of HEXA ins TATC in rabbits was determined by Sanger sequencing (Supplementary Fig. S5), qRT-PCR (Fig. 1h), and western blot (Fig. 1i). The results showed a significantly reduced HEXA in HEXA ins TATC rabbits compared with WT controls. The typical phenotypes of the increasingly frequent of head raising, convulsions (Supplementary Fig. S6a and Movies S1, S2), abnormal gait with decreased sway length (Supplementary Fig. S6b and Movies S1, S2), claspings of the limbs, and increased cervical lordosis (Fig. 1i), muscle fibrosis (Fig. 1k) and enlargement of perineural space (Fig. 1l) were also determined in HEXA ins TATC rabbits when compared with WT controls. These phenotypes were similar with late-onset or chronic adult gangliosidosis in TSD patient exhibiting as limb-girdle weakness, followed by the development of ataxia and progressive neuromuscular weakness binding site.

In summary, this study for the first time verified the feasibility of PE system-mediated base insertions, deletions, and conversions in rabbit. This ideal and novel HEXA ins TATC rabbit model would be beneficial for the pathogenic mechanism study and drug screening to treat TSD in the future.

Acknowledgements
We thank Peiran Hu and Nannan Li for assistance at the Embryo Engineering Center for critical technical assistance. This study was financially supported by the National Key Research and Development Program of China Stem Cell and Translational Research (2019YFA0110700). The Strategic Priority Research Program of the Chinese Academy of Sciences (XDA16030501, XDA16030503), Key Research & Development Program of Guangzhou Regenerative Medicine, and Health Guangdong Laboratory (2018GZ01104004).

Author contributions
Y.Q., L.L., and T.Z. conceived and designed the experiments. Y.Q., D.Z., and T.S. contributed reagents/materials/analysis tools. Y.Q. and D.Z. wrote the paper. All authors have read and approved the final manuscript.

Conflict of interest
The authors declare no competing interests.

References
1. Kolodny, E. H. Molecular genetics of the beta-hexosaminidase isoenzymes: an introduction. Adv. Genet. 44, 101–126 (2001).
2. Frisch, A. et al. Origin and spread of the 1278insTATC mutation causing Tay-Sachs disease in Ashkenazi Jews: genetic drift as a robust and parsimonious hypothesis. Hum. Genet. 114, 366–376 (2004).
3. Phaneuf, D. et al. Dramatically different phenotypes in mouse models of human Tay-Sachs and Sandhoff diseases. Hum. Mol. Genet. 5, 1–14 (1996).
4. Jasin, M. & Haber, J. E. The democratization of gene editing: Insights from site-specific cleavage and double-strand break repair. DNA Repair 44, 6–16 (2016).
5. Anzalone, A. V. et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576, 149–157 (2019).
6. Rickmeier, T. et al. GMI2 gangliosidosis in an adult patient. J. Comp. Pathol. 148, 243–247 (2013).
7. Wang, Y. et al. Generation of knockout rabbits using transcription activator-like effector nucleases. Cell Regen. 3, 3 (2014).
8. Landrum, M. J. et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res. 44, D862–D866 (2016).
9. Brinkman, E. K., Chen, T., Amendola, M. & van Steensel, B. Easy quantitative assessment of genome editing by sequence trace decomposition. Nucleic Acids Res. 42, e168 (2014).
10. Myerowitz, R. & Costigan, F. C. The major defect in Ashkenazi Jews with Tay-Sachs disease is an insertion in the gene for the alpha-chain of beta-hexosaminidase. J. Biol. Chem. 263, 18587–18589 (1988).
11. Jeyakumar, M. et al. An inducible mouse model of late onset Tay-Sachs disease. Neurobiol. Dis. 10, 201–210 (2002).