Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype

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We demonstrate CRISPR-Cas9–mediated correction of a Fah mutation in hepatocytes in a mouse model of the human disease hereditary tyrosinemia. Delivery of components of the CRISPR-Cas9 system by hydrodynamic injection resulted in initial expression of the wild-type Fah protein in ~1/250 liver cells. Expansion of Fah-positive hepatocytes rescued the body weight loss phenotype. Our study indicates that CRISPR-Cas9–mediated genome editing is possible in adult animals and has potential for correction of human genetic diseases.

The type II bacterial clustered, regularly interspaced, palindromic repeats (CRISPR)-associated (Cas) system has been engineered into a powerful genome editing tool consisting of the Cas9 nuclease and a single guide RNA (sgRNA)1–4. The sgRNA targets Cas9 to genomic regions that are complementary to the 20-nucleotide (nt) target region of the sgRNA and that contain a 5′-NGG-3′ protospacer-adjacent motif (PAM). Double-stranded DNA breaks generated by Cas9 at target loci are repaired by nonhomologous end-joining or homology-directed repair (HDR). CRISPR-Cas9 genome editing has been applied to correct disease-causing mutations in mouse zygotes and human cell lines for cataract5 and cystic fibrosis6, but delivery to adult mammalian organs to correct genetic disease genes has not been reported to our knowledge.

To investigate the potential of CRISPR-Cas9–mediated in vivo genome editing in adult animals, we used a mouse model of hereditary tyrosinemia type I (HTI), a fatal genetic disease caused by mutation of fumarylacetoacetate hydrolase (FAH), the last enzyme in the tyrosine catabolic pathway (Supplementary Fig. 1a)5,6. The Fah5981SB mouse model8,9 (referred to here as Fahmut/mut) of HTI harbors the same homozygous G→A point mutation of the last nucleotide of exon 8 as causes the human disease. This mutation causes skipping of exon 8 during splicing and formation of truncated, unstable FAH protein (Fig. 1a). FAH deficiency causes accumulation of toxic metabolites, such as fumarylacetoacetate, in hepatocytes, resulting in severe liver damage8, 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), a pharmacological inhibitor of the tyrosine catabolic pathway upstream of FAH, rescues the phenotype and prevents acute liver injury4. A previous study showed that targeted integration by homologous recombination of adeno-associated virus carrying the wild-type Fah sequence could achieve stable gene repair in vivo in mice, but required multiple rounds of NTBC withdrawal and recovery8. Liver cells in which Fah has been repaired have a selective advantage and can expand and repopulate the liver8. Diseases in which positive selection of corrected cells occurs8,9 may be particularly suitable for gene repair–based therapy; indeed, repair of 1/10,000 hepatocytes was reported to rescue the phenotype of Fahmut/mut mice8.

To edit the endogenous Fah locus, we individually cloned three sgRNAs targeting Fah (FAH1, FAH2 and FAH3) (Supplementary Methods) into the pX330 vector10, which co-expresses one sgRNA and Cas9 (Supplementary Fig. 1b–d). To facilitate homologous recombination and correct the G→A splicing mutation, a 199-nt, single-stranded DNA (ssDNA) donor was synthesized harboring the wild-type G nucleotide and homology arms flanking the sgRNA target region (Fig. 1a and Supplementary Tables 1 and 2). Adult Fahmut/mut mice were given hydrodynamic tail vein injections11 with (i) saline, (ii) the ssDNA oligo alone, (iii) ssDNA oligo plus pX330 expressing Cas9 only (‘unguided Cas9’) or (iv) ssDNA oligo plus pX330 expressing Cas9 and one of the sgRNAs (FAH1–3). Fahmut/mut mice injected with saline, ssDNA oligo alone or unguided Cas9, and kept without NTBC-containing water, rapidly lost 20% of their body weight and had to be euthanized (Fig. 1b and Supplementary Fig. 2). Mice receiving oligo plus pX330 expressing Cas9 plus FAH2 did not lose weight, and weight loss in FAH1- and FAH3-treated mice was <20% after 30 d without NTBC water. Mice treated with FAH1 or FAH3 were put back on NTBC water for 7 d and subjected to a second round of NTBC withdrawal for 28 d; these mice regained all the weight they had lost (Fig. 1c). Liver damage was substantially less in FAH2-treated mice at 30 d off NTBC water compared to untreated Fahmut/mut mice that were not receiving NTBC water, as indicated by liver histology (Fig. 1d) and serum markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin8 (Fig. 1e–g), suggesting a functional rescue of the Fah deficiency–induced liver damage.

To see whether Cas9-mediated genome editing generates Fah+ hepatocytes in vivo, we examined the liver tissue of treated mice by immunohistochemical staining with an Fah-specific antibody. Thirty-three days after treatment with FAH2 and 30 d without NTBC water,

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Fahmut/mut mice had widespread patches of Fah+ hepatocytes (33.5% ± 3.3%, n = 3 mice) (Fig. 2a and Supplementary Fig. 3a). To measure the initial Fah gene repair frequency, we treated Fahmut/mut mice with FAH2 and kept them on NTBC water (to prevent positive selection of corrected cells) for 6 d before euthanizing them. As shown by immunohistochemical staining of Fah+ cells, the initial repair frequency was 0.40 ± 0.12% (n = 3 mice) for mice treated with FAH2 compared to 0.01 ± 0.02% for those with unguided Cas9 (Fig. 2a). We also performed deep sequencing to examine the initial repair rate; however, due to the error rate of sequencing, the mixture of nonparenchymal cells and polyploidy of hepatocytes, this approach could not detect low-frequency single-nucleotide polymorphisms in hepatocytes11.

We also carried out RT-PCR using primers spanning Fah exons 5–9 to determine whether the Fah splicing mutation was corrected in the liver. We found that wild-type mice had a 405-bp PCR band corresponding to the truncated Fah mRNA lacking exon 8 and Fahmut/mut mice injected with FAH1, 2 or 3 had both the 305- and 405-bp PCR bands, indicating that the exon 8 to exon 9 splicing is restored in a subset of hepatocytes (Fig. 2b). Sequencing of the 405-bp bands in CRISPR-Cas9 treated mice confirmed that the corrected G nucleotide is included in the PCR product (Fig. 2c). Quantitative RT-PCR using primers spanning exons 8 to 9 on liver samples from CRISPR-Cas9–treated mice showed they had Fah mRNA at 8–36% of levels in wild-type mice at the end of NTBC water withdrawal (Fig. 2d). These levels are consistent with the proportion of Fah+ hepatocytes detected by immunohistochemistry (Fig. 2a and Supplementary Fig. 3a) and the A→G correction rate (~9%, n = 2) detected by deep sequencing in FAH2-treated livers at 30 d off NTBC (Supplementary Fig. 3b–d and Supplementary Table 3). We note that an indel rate of ~26% was also detected by sequencing of these samples (Supplementary Fig. 3b–d and Supplementary Table 3); further work will be needed to assess the initial rate of indel formation compared to gene correction.

To examine potential off-target effects, we used a published prediction tool2 to identify potential off-target sites in the mouse genome for FAH1, 2 and 3 (Supplementary Figs. 4–6). In mouse 3T3 cells transfected with FAH 1, 2, 3 or control (unguided Cas9), the editing at the Fah locus and three or four potential off-target sites was measured using the mismatch-specific Surveyor nuclease assay1. Cleavage was detected at Fah in 3T3 cells, indicating that the one nucleotide mismatch between FAH1, 2 and 3 and the wild-type Fah gene does not prevent Cas9–mediated editing (Supplementary Figs. 4–6). Cleavage was not detected at the assayed three to four top-ranking off-target sites.
for each sgRNA (Supplementary Figs. 4–6). The PCR products from three off-target sites of FAH2 were sequenced, and <0.3% indels were detected (Supplementary Fig. 5c and Supplementary Table 4). Wild-type FVB mice injected with Cas9 plus sgRNA showed no body weight loss, no signs of hyperplasia and extremely low Cas9 expression after 3 months (Supplementary Figs. 7 and 8), suggesting that hydrodynamic injection of these components was well-tolerated.

In summary, these data demonstrate the potential to correct disease genes in vivo in adult mouse liver using a CRISPR-Cas9 system. Transient expression of Cas9, sgRNA and a co-injected ssDNA by non-viral hydrodynamic injection is sufficient to restore the weight loss of a mouse model of HTI. The strong positive selection and expansion of Fah+ hepatocytes in the Fahmut/mut liver may have contributed to the correction of the disease phenotype, given the observed initial genetic correction rate of ~1/250 cells. We note that the initial efficiency of repair by subsequent work to correct hemophilia in mice12. We also note that the initial efficiency of repair will be required for its broad therapeutic application. Therefore, improvements to CRISPR delivery methods and hydrodynamic injection is unlikely to be used for clinical implementation. This proof-of-principle study indicates that correction of genetic disease in vivo with CRISPR-Cas9 may be possible, and we believe that recent advances in the delivery of nucleic acid therapeutics provide hope that CRISPR-Cas9-mediated correction may be translatable to humans13.

Accession codes. BioProject: PRJNA242331.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

H.Y., W.X. and D.G.A. designed the study. H.Y., W.X. and D.G.A. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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Corrigendum: Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype

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In the version of this article initially published online, in the legend for Figure 1d, the scale bars are 100 μm and 20 μm, not “mm.” The error has been corrected for the print, PDF and HTML versions of this article.